STUDIES WITH THE AGGREGATION PHEROMONES OF *RYHZOPERTHA DOMINICA* (COLEOPTERA: BOSTRICHIDAE): HABITAT AFFINITIES, SEASONAL FLIGHT ACTIVITY, AND PHEROMONE-MEDIATED HOST SELECTION BEHAVIOR

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2005

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ACKNOWLEDGEMENT

Foremost, I give thanks to God Almighty for the opportunity to enjoy good health throughout the course of my PhD program.

The contributions of my academic committee members are gratefully acknowledged. I thank my major advisor Dr. T. W. Phillips for his support, constructive criticisms, and editing. My gratitude goes to Dr. M. E Payton for putting up with my constant flow of emails and phone calls for statistical advice, and to Dr J.W. Dillwith and Dr. P. G. Mulder for their supervisions and professional encouragement during this time.

Special thanks to my wife Titilade for her continuous love, encouragement, and prayers. I thank Mrs. Oluyemisi Olukoya for making available the use of her laptop computer when mine decided to take a "break" at a critical stage in my dissertation writing.

Thanks also to Dr. Wendell Burkholder, USDA, Madison, WI (retired) and Alain Van Rhyckenham, Insect Limited Inc., Westfield, IN for providing synthetic *Rhyzopertha dominica* and *Prostephanus truncatus* pheromones, respectively.

Many other faculty and staff in the Department of Entomology have contributed one way or another to the success of my PhD program. One of these wonderful individuals is Dr. Kris Giles, with whom I had useful discussions relating to my research, and science in general. Kris allowed me unrestricted access to facilities in his laboratory. Dr. Richard Berberet was always friendly, generous with microscopes, and more importantly, always willing to spare some of his valuable time whenever I needed

professional assistance. Dr Richard Grantham offered technical help whenever I had difficulties with computers and related matters. Phillip Taylor graciously helped with identification of trees at my experimental sites. Don Arnold spent a great deal of time identifying hundred of non-target insects captured in my pheromone-baited traps. Edmond Bonjour is always willing to share his vast knowledge of stored-products with me, and ensured timely supply of materials needed for my research. James Robertson provided dedicated and efficient technical assistance with counting of insects and gas chromatography work. To all and each one of you, I say a big thank you.

I want to acknowledge Dr. Tom Phillips and the Department of Entomology and Plant Pathology for the award of a Graduate Research Assistant with which my PhD program was financed.

In addition, the financial contribution from USDA-CSREES Risk Avoidance and Mitigation Program and the Oklahoma State University Integrated Pest Management Mini-Grant Program are gratefully acknowledged.

Finally, I would like to offer my apologies to those individuals whom I might have inadvertently failed to mention, but that must have contributed in one way or another to the success of my PhD program. You have all been wonderful and I am grateful.

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CHAPTER I

INTRODUCTION

Stored Grain Insects Pests

Grain is stored primarily to increase the net return by holding grain until prices are more favorable (Anderson et al. 1995). However, storing grain may result in overall loss of quality of the commodity, thereby decreasing potential economic returns. Risks to stored grains often result from direct feeding by several species of insects that reduce grain weight, nutritional value, and germination. Infestation also causes contamination, odor, mold and heat-damage problems that reduce the quality of the grain making it unfit for consumption by humans and livestock. On a worldwide basis, post-harvest losses of durable crops are estimated at over 10% (Aidoo 1993, Harein and Meronuck 1995), most of which are attributed to insect pest damage. The situation is more precarious in tropical developing countries where post-harvest losses have been estimated at over 20% (Aidoo 1993), aggravated by inclement weather conditions and poor storage technology.

Stored grain insect pests are found mainly within two insect orders, Coleoptera and Lepidoptera. Species in the order Coleoptera are commonly referred to as beetles or weevils, and are easily recognized by their forewings, which are modified into hard elytra, covering the dorsal surface in a straight mid-dorsal line. Beetle forewings are not used for flight. The mandibles may become very large in the males of some species, while others have prominent development of their heads. The Lepidoptera commonly referred to as butterflies or moths are recognized by their scaly membranous forewings. Adults have large antennae and an extendable feeding tube (proboscis). Adult Lepidoptera found in grain storage do not feed; damage is created by the immature (often called caterpillars) larvae, which are equipped with mandibulate mouthparts.

Based on their feeding habit, stored grain pests may be classified into two broad groups, external and internal feeders, commonly referred to as secondary and primary insect pests, respectively. The primary and secondary designation does not imply more or less importance, but more appropriately indicates the order of succession in the infestation process of whole grain. External-feeders feed mainly on milled products and broken grains. The larvae of these insects may also consume the germ, bran and endosperm of intact grain. Economically important external feeders in the order Coleoptera include *Tribolium* (Tenebrionidae), *Cryptolestes* (Laemophloeidae) and *Oryzaephilus* (Silvanidae). The Indianmeal moth (*Plodia interpunctella* Hübner), the almond moth (*Cadra cautella* (Walker)) and the Mediterranean flour moth *(Ephestia kuehniella* Zeller.) are important external-feeding pests in the order Lepidoptera (family Pyralidae). Internal feeders feed on whole, sound grain and larvae develop inside grain kernels. This group of insects constitutes the most serious economic pests because their cryptic feeding habit makes infestations difficult to detect until progeny emerge. Additionally, feeding habits of internal feeders can produce "insect damage kernels" or IDK (Federal Grain Inspection Service 1997). A wheat consignment containing more than 32 IDK per 100 g is designated a sample grade (FGIS 1997). Sample grade wheat cannot be sold for human consumption and market value drops dramatically (Flinn et al. 2004). The *Sitophilus* weevils, Angoumois grain moth *Sitrotoga cereallela* (Oliv.), seed beetles (family Bruchidae) and three species in the family Bostrichidae (next section) are examples of economically important internal grain feeders worldwide.

Distribution and Economic Importance of Stored-Product Bostrichidae

Bostrichidae, commonly referred to as bostrichids beetles, comprise of about 550 species in 99 genera of which 77 species in 26 genera occur in North America (Ivie 2002a Ivie 2002b, Marske and Ivie 2003). They vary in size, are elongate, cylindrical in cross-section, the head is invisible when viewed from above and they are red brown to dark brown in color. Members of the family live mainly on dead and dried woods, and are recognized as pests of timber (Potter 1935, Fisher 1950, Ivie 2002a, Ivie 2002b). Bostrichids closely resemble and are often mistaken for bark and ambrosia beetles in the family Scolytidae but may be easily distinguished from the Scolytidae by their tuberculate and rasplike pronotum, straight instead of elbowed antennae with a three or four segmented club, and by their five segmented tarsi (Fisher 1950). Descriptions and keys to identification of species belonging to the family Bostrichidae can be found in Fisher (1950) and Ivie (2002a).

Important stored product Bostrichidae pests are *Dinoderus* spp, the larger grain borer, *Prostephanus truncatus* (Horn.) and the lesser grain borer, *Rhyzopertha dominica* (F.). Four species of Dinoderus, namely *D. bifoveolatus* (Wollaston), *D. minutus* (F.), *D. porcellus* Lesne, and *D. oblongopunctatus* Lesne are currently restricted to tropical Africa where they are regarded as important pests of maize, *Zea mays* L. (Gramineae) and cassava, *Manihot esculenta* Crantz (Euphorbiaceae) (Schäfer et al. 2000, Borgemeister et al. 1999). However, the bamboo borer *D. minutus* is believed to have been introduced into the southern USA, particularly in Louisiana and Florida (Cotton 1950). *P. truncatus* is currently endemic to México and Central America, from where they were accidentally introduced into Africa in the early 1980s, where they cause

extensive damage to stored maize and cassava (Borgemeister et al. 1999, Kumar 2001). There are reports of the possible occurrence of *P. truncatus* in southern states of the United State of America (USA) (Cotton 1950, Gorham 1987), but results from three a year trapping study using both synthetic and natural pheromones of *P. truncatus* suggested that this pest is not presently found in Stillwater, OK (Edde and Phillips 2005a), and thus may be considered a quarantine pest in Oklahoma.

Although, *R. dominica* was described by Fabricius in 1792 from specimens taken from nuts and roots in South America (Cotton 1950), the native home of this species is believed to be the Indian subcontinent (Schwardt 1933, Potter 1935). The belief that India or its neighboring region is the possible origin for *R. dominica* is reinforced by the fact that this location is the focus of a large number of species of Bostrichidae (Schwardt 1933, Potter 1935). Today, *R. dominica* is today found as a pest of stored grain in warmer regions of the world lying in the belt between latitude 40° N and 40° S of the equator. This insect feeds on a wide variety of food materials, but achieves its maximum reproductive success on dry grain in the family Gramineae. *R. dominica* was first noticed in the USA around 1861 (Leconte 1862) and became established in the country in early 1920's (Back and Cotton, 1922), perhaps augmented by importation of *R. dominica* infested wheat from Australia (Doan 1919). The insect is now considered one of the most damaging pests of stored wheat in the USA (Hagstrum et al. 1999) because of the ability of adults and larvae to utilize whole, sound grain, and to survive in grains with very low moisture content (<10%) and at cold temperature (as low as 13°C). Adults are also long-lived, strong fliers and are capable of infesting a stored grain without being directly introduced from a contaminated source. Susceptible cereal crops include wheat,

maize, rice and sorghum (Haines 1991, Hagstrum et al. 1999). *R. dominica* infested kernels are often riddled with holes and surrounded by powder resulting from boring and feeding activities of adults and larvae; resulting in substantial economic loss. *R. dominica* may impair end-use quality of infested grain. Sanchez-Manrinez et al. (1997) observed that flours from wheat infested with *R. dominica* have poor baking properties.

Current management strategies for *R. dominica* involve the use of broad-spectrum insecticides, particularly organophosphates or pyrethroid grain protectants such as malathion, pirimiphos-methyl, chlorpyrifos-methyl, and deltamethrin (Arthur 1996). Insecticides are effective in many cases, but insecticide resistance is evident in many populations of *R. dominica,* due to excessive use (Yao and Lo 1994, Benhalima et al. 2004). Additionally, insecticides can be harmful to nontarget species and may pollute the environment **(**Lorini and Galley 1999). Many of the insecticides used by the cereal foods industry are being lost due to insecticide resistance or regulatory changes. Thus, there is a need to develop sustainable and environmentally-friendly pest management tactics.

Biology of *R. dominica*

Life History

Adult *R. dominica* is about 2-3 mm in length and 1 to 1.2 mm in width. The insect is long lived. Adults fed *ad libitum* on wheat *Triticum aestivum* L. (Poaceae) can live for 54 weeks at 30°C and 66% RH (Edde unpublished data).

Eggs are oval-shaped, 0.5-0.6 mm in length and 0.2 in diameter (Potter 1935, Thompson 1966). One female can deposit between 200-500 eggs during her lifetime. Eggs may be deposited either in clusters on grain, or singly among the frass produced by

the insect. The beetle lays an average of one to seven eggs per day over several months (Hagstrum and Flinn 1994), and the number of eggs laid may be affected by photoperiods, such that more eggs are laid during long photoperiod (Aslam et al. 1994). Larval hatch takes between 5-14 days depending on environmental conditions (Chittenden 1911, Crombie 1941). First instar larvae are campodeiform and usually bore into the kernel where it remains and continues to feed on the endosperm until it becomes an adult. The second larval stage is scarabaeiform but is capable of active locomotion. The third and subsequent larval stages are also scarabaeiform, and are largely immobile. The number of molts may range from four to five (Potter 1935) or even six to seven (Howe 1950) depending on environmental conditions. *R. dominica* requires about 27-30 days at 30°C and 60% RH to develop from egg to adult when reared on wheat (Edde unpublished data).

Sex Differences

No consistent recognizable external sex differences exist between male and female *R. dominica*. Stemley and Wilbur (1966), working with a Kansas strain, suggested that the last (fifth) ventral abdominal sternite of female *R. dominica* is pale yellow whereas those of males are uniformly brown. However, Singh and Liles (1972) and Cline (1973) considered the use of color unreliable to separate *R. dominica* sexes. Ghorpade and Thyagarajan (1980) and Bashir (2000), suggested the existence of a distinct transverse, punctuate groove on the fifth abdominal sternite of the male from Pakistan, which is never present in females. However, Sinclair (1981) did not find the punctuate groove in *R. dominica* strains from Queensland, Australia. Similarly, the punctuate groove is not

easily discernible in laboratory reared or field-collected *R. dominica* in central Oklahoma, USA. (Edde personal observation). Further studies are required to characterize and document superficial differences, if any, among *R. dominica* strains from different geographical regions. Since most insect pests of stored products are repeatedly transported around the world by commerce, the use of such external characters may prove useful in identification of possible origins of infestation. An alternative method is the "squeezing" method proposed by Crombie (1941). In this method, the abdomen of live or freshly killed specimen is gently squeezed to cause extrusion of their genitalia, which were then viewed under a dissecting microscope. This method is accurate, not deleterious and consistent for different strains of *R. dominica* (Crombie 1941, Singh and Liles 1972).

The difficulties associated with the use of external characters to separate *R. dominica* sexes may be circumvented by sexing the beetles at the immature stage using sexual dimorphism of the pupal stage (Potter 1935). Female pupae can be easily distinguished by the presence of two to three segmented papillae projecting from the abdominal segments, whereas at the end of the abdomen of male pupae is a pair of two-segmented structures fused to the abdomen for their whole length (Potter 1935). A major setback to sexing the pupal stage is that it is found within whole grain kernels, thus making collection of insects for sexing difficult. However, this problem can be minimized by rearing pupae on wheat flour and transferring sexed pupae on kibbled wheat with particle size in the range 1.4-2mm (Longstaff and Starick 1989).

Rhyzopertha dominica Flight Activity

R. dominica is a crepuscular species that has a small peak of flight activity at sunrise, a large flight response at sunset, and little or no flight activity during the night (Leos-Martinez et al. 1986, Wright and Morton 1995). Greatest flight activity occurs late in the evening, 2-3 hours before sunset (Wright and Morton 1995, Sinclair and Haddrell 1985). There are no differences in flight activity between male and female beetles; however, age may affect flight initiation, such that young adults (between 3-6 days old) have a greater tendency to initiate flight activity than older beetles (Aslam et al. 1994).

Optimum temperature for flight initiation varies and depends on geographical populations. In the laboratory, Dowdy (1994) found that 19.9° and 44.6°C were the minimum and maximum temperature threshold, respectively, for flight activity in USA populations. In Australia, 16°C and 37°Cwere found to be the lower and upper temperature thresholds for flight activity (Wright and Morton 1995). Unlike temperature, humidity does not have a significant effect on *R. dominica* flight activity under laboratory conditions (Dowdy 1994, Wright and Morton 1995).

R. dominica is a strong flier (Winterbottom 1922, Cotton 1950). The beetle has been trapped in diverse environments, including woodlands that are substantial distances from grain stores, during warm months (Cogburn 1988, Throne and Cline 1994, Edde et al. 2005b). Outdoor trapping studies have shown a characteristic daily and seasonal activity pattern for *R. dominica* which appears to be determined by weather conditions (Sinclair and Haddrell 1985, Throne and Cline 1994, Edde et al. 2005b). The importance of dispersal in *R. dominica* population dynamics has been largely ignored when formulating integrated pest management (IPM) programs for stored grain. This is

because, although speculated, very little is known on dispersal between *R. dominica* metapopulations. Secondly, unlike studies on flight behavior of the insect in the laboratory, too little is known about the factors that influence flight initiation, migration and dispersal of the pest in non-grain habitats. Finally, testing the impact of this phenomenon on *R. dominica* populations in agricultural settings, such as grain elevators, and non-agricultural settings is cumbersome and difficult. However, these kinds of studies are essential for accurate models of insect dispersal to enable realistic forecasting of pest pressure on stored grains, and this is not possible without detailed knowledge of insect flight behavior and ecology.

Feeding Ecology of *R. dominica*

It is believed that contemporary stored product Bostrichidae were originally xylophagous, but became facultatively associated with stored grain (Potter 1935). The deflexed head and strong mandibles of *R. dominica*, *P. truncatus* and *Dinoderus* spp are typical of wood boring beetles. The large pronotum offers protection to the beetles while tunneling and provides support for the mandibular muscles. Most wood or twig borers receive their actual nutrition from the starch content in the wood they consume, therefore making the switch to a stored grain product understandable. Many of the present-day stored product insect pests are known to have undergone behavioral changes in their food choices, and probably adjustment to a new environment (Linsley 1944). In species in the family Bruchidae, such adaptations may have represented little or no change in food habits and probably little adjustment to this new environment. Many bruchid beetles breed in seeds and have been collected from pods of indigenous leguminous trees such as

Acacia species. However, for some species, their present lack of occurrence on wild plants could be attributed to the loss or reduction of original host plants or host plant habitat, or inability to effectively feed on seeds of wild plants following adaptation for use as domesticated plants.

R. dominica is reportedly highly polyphagous, and has been recorded feeding or breeding on seeds of legumes (e.g. chickpeas, peanuts, beans), tubers (potato), bulbs, roots (cassava) and cereals (e.g. rice, wheat, sorghum, pearl millet, malt barley) (Potter, 1935, Linsley, 1944, Mathew, 1987). Also reported are packaging material made from wood and several forest tree seeds (e.g. Potter 1935, Wright et al. 1990). However, the majority of the plant species reported by Potter (1935) as possible hosts for *R. dominica* may be considered speculative, as there are few or no experimental data to support these claims. In attempts to elucidate the connection between the wild and grain storage habitats in *R. dominica*, Wright et al. (1990) found that the insect was able to feed and reproduce on several fruits and seeds collected from the forest. Morrison et al. (2005) found that *R. dominica* was able to penetrate shells of pecan [*Carya illnoinensis* (Wang.) Koch.] in the laboratory, but reproduction was marginal on pecan kernels. It is possible, therefore, that wild habitats may serve as temporary niches or alternate food sources for *R. dominica* during the absence of preferred hosts like wheat, maize and rice.

The complexity of the food range of *R. dominica* has been a major challenge in studying the nutritional ecology of the species. Most of the decisions and responses that an insect makes during its life occurs within a nutritional context (Slansky 1982). There are consequences for choosing the wrong host in the form of adult mortality, and reduced fitness resulting from the inability to maintain the ideal value for life and life history

parameters (Elkinton et al. 1980, Slansky 1982, Langor et al. 1990, Zvereva 2002, Helms and Hunter 2005)). Knowledge about the dietary information of an organism can shed light on a variety of functions and patterns such as food allocation, feeding behavior, habitat preference and ecological aggregation of the organism. Little published information is available on the effects of host plant on the reproductive success and production of aggregation pheromones in *R. dominica*. Recently Bashir et al. (2003a) found that pheromone release rates in *R. dominica* were lowered when male signaler was moved from a suitable host to an unsuitable host, but release rate were restored when the move was reversed. Because *R. dominica* is highly polyphagous, it is pertinent to broaden studies on feeding/nutritional ecology of *R. dominica* that may yield information that may allow crop breeders to effectively manipulate the agrosystem (such as development of resistant varieties that are difficult to feed upon and/or impair pheromone production) to disrupt the normal performance of the pest (Slansky 1982).

Aspects of Chemical Ecology of *R. dominica*

Definitions

The term *ecology* was coined in 1869 by the German biologist Ernst Haeckel from two Greek words: *oikos*, meaning "house" or "place to live" and *logos*, meaning study. Ecology, therefore, can be defined as the study of how organisms interact with each other and their physical environment. Chemical ecology is a specific aspect of ecology restricted to chemicals (semiochemicals) that mediate interactions between living organisms (Byers 1989). It focuses on the production of and response to signaling molecules, toxins, and other organic compounds.

The term “semiochemicals’ is used to describe chemicals that convey information between organisms. Two groups of semiochemicals are allelochemicals and pheromones. The underlying factor in this classification is distinguishing the receiver from the sender. Allelochemicals and pheromones are used for interspecific and intraspecific communications, respectively. Two commonly utilized pheromones among the beetles, as well as in other insect orders, are sex and aggregation pheromones. Sex pheromones have been traditionally considered as substances released by individuals of one sex to attract members of the opposite sex, resulting in location of the emitter and subsequently mating. Aggregation pheromones may be produced by one or both sexes to increase the density of conspecifics near the pheromone source for feeding, mating and protection. Thus, the biological and ecological significance of pheromone is their species specificity; however, cross-species attractions are known to occur, which may or may not benefit the releaser. Allelochemicals may further be classified into kairomones and allomones according to the beneficiary of the interaction. Allomone signaling benefits the emitter, while kairomone release benefits the receiver. When synomones are used both sides benefit.

The word “pheromone” is coined from two Greek words, *pherrein,* meaning to carry, and *hormon*, meaning to excite. The first insect pheromone to be identified was that of the silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae); the compound was found to be (*E*10, Z12)-hexadecadien-1-ol, and called “bombykol” (Butenandt et al. 1959). Since then, pheromones have been identified from over 1600 species of insects in over 90 families in nine orders, of which about 51 and 21% of the families represented are form Lepidoptera and Coleoptera, respectively (Mayer and Mclaughlin 1991). The

ease of rearing and/or working with Lepidoptera species may explain greater investigations that have been devoted to the pheromone chemistry in the order, relative to other insect orders. The importance of pheromone in chemical communication among insects, and theist potential use for insect control was recognized as early as 1882 (Roelofs 1995).

Just after the discovery of bombykol, it was generally thought that each insect species produced and responded to a single pheromone (Karlson and Butenandt 1959). However, (Silverstein et al. 1966) found that *Ips paraconfusus* Lanier (Coleoptera: Scolytidae) produce and respond to a blend of three pheromones (e.g., (*S*)-(-)-ipsenol, (*S*)-(+)-ipsdienol, and (4S)-*cis*-verbenol). It has since been discovered that most insects produce multi-component blends of pheromones, and that concept of single component system is the exception rather than the rule. The blend of pheromones is important because some or all components may act as synergists i.e. individually they elicit little or no attractiveness, but together they are highly attractive. However, redundancy in the pheromone signal is a common feature in many insect species. Redundancy may occur when pheromone components are equally attractive or the presence of more than one pheromone component in the blend did not increase attraction (Linn et al. 1984, McBrien et al. 2002).

Chemical Nature of Insect Pheromones

Pheromones are the primary method for long distance communication in insects (Roelofs 1995). To have sufficient volatility, airborne pheromone molecules may be limited to 5 to 20 carbons and a molecular weight of 80 - 300 daltons (Wilson 1980). Above 20 carbons and a molecular weight of 300 daltons , pheromone molecules become

greatly diverse, less volatile, and relatively more expensive to synthesize and transport by the insect (Wilson 1980). Pheromone compounds with high molecular weight are less volatile and tend to be effective in attraction and stimulation when prolonged exposure is necessary (Tumilson and Teal 1987). Examples of these types of compounds include host marking/oviposition deterrent pheromones found in species of Tephritidae and *Daiops caustonae* (Diptera: Lonchaeidae) (Causton and Rangel 2002).

Compounds commonly used for intraspecific communication among insects are low molecular weight acids, alcohols, esters, aldehydes, ketones, epoxides, lactones, terpenes and sequiterpenes (Table 1). To achieve species specificity, insects have evolved several strategies in chemical communication. Closely related species may use a set of similar chemical substances, but in different ratios in each species, or use the same pheromone compounds, but different additional compounds in each species. In other cases, species may be producing entirely different compounds as pheromones. Thus, by utilizing different compounds or blends, species with similar pheromone systems are reproductively isolated, either temporarily, or geographically.

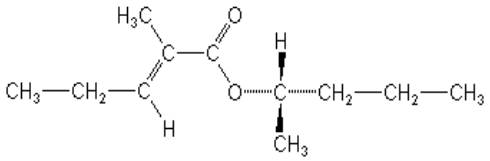
When compared with pheromones of Lepidoptera, which are largely straight chain alcohols, aldehydes and acetates, Coleoptera have evolved complex structural diversity commensurate with the orders phylogenetic diversity (Tillman et al. 1999). Pheromone structures used by Coleoptera vary from isoprenoids in bark beetles (Tumlinson and Teal 1987, Barkawi et al. 2003), and fatty acid derived (*R*)-(+)-4-methyl-1-nonanol used by *Tenebrio molitor* L. (Tenebrionidae) (Tanaka et al. 1986, Islam et al. 1999), lactone derived (R,Z)-5-(-)-(oct-1-enyl)-oxacylopentan-2-one (japonilure) used by *Anomala cuprea* Hope (Scarabaeidae; Leal 2001) to amino acid derived L-Leusine

methyl ester produced by female *Phyllophaga lanceolata* (Say) (Scarabaeidae) (Nojima et al. 2003). However, a generic theme of structural types exists within groups as evidenced by the use of same structurally related compounds by many species of the same genus, resulting in the development of common biosynthetic pathways (Tumlinson and Teal 1987). Pheromones in Coleoptera therefore are either, (1) modifications of host compounds, (2) sequestered from host compounds that are slightly modified, (3) synthesized *de novo* (i.e. effectively built from scratch from precursors) or (4) synthesized by microorganisms residing in the gut of insects that are capable of converting dietary chemicals into semiochemicals.

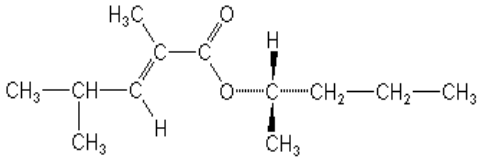
Pheromone Biology of *R. dominica*

The only three species in the family Bostrichidae for which pheromones have been identified are *D. bifoveolatus*, *P. truncatus* and *R. dominica* (Khorramshahi and Burkholder 1981, Williams et al. 1981, Hodges et al. 1984, Cork et al. 1991, Tolasch et al. 2002). In general, pheromones of the Bostrichidae are relatively simple compounds containing 9-12 carbon, and are undoubtedly not sequestered from the host (Birkinshaw 1998).

Similar to *P. truncatus* and *D. bifoveolatus, R. dominica* produces aggregation pheromones that were reported as (S)-(+)-1-methylbutyl (*E*)-2-methyl-2-pentenoate and (S)-(+)-1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate, commonly called Dominicalure-1 (or DL1) and Dominicalure-2 (or DL2), respectively (Williams et al. 1981, Khorramshahi and Burkholder 1981).



Dominicalure -1



Dominicalure-2

Fig.1 Aggregation pheromones of *R. dominica*

Dominicalure-1 (DL-1) and Dominicalure-2 (DL-2) were isolated and identified from volatiles collected from mixed populations of male and female beetles or males alone (Khorramshahi and Burkholder 1981). Williams et al. (1981) found that beetle-produced (+) isomers were twice as active as synthetic isomers.

Biosynthesis of *R. dominica* aggregation pheromones is not well understood (Vanderwel and Oehlschlager 1987), but it has been shown that feeding is an obligatory prerequisite (Mayhew 1994). The food source also serves as a source of oviposition and is consumed by developing larvae and adults. It is believed that feeding triggers the acetogenic pathways from the biosynthetic precursors by removing inhibition of the corpora allata and triggering release of juvenile hormone (Vanderwel and Oehlschlager 1987, Landolt and Phillips 1997). Juvenile hormone activates brain neurosecretory cells to release a stimulatory brain hormone that drives pheromone synthesis (Hughes and Renwick 1977). The requirement for food resources as a prerequisite for pheromone production has been demonstrated in insect species in several families including the Curculionidae (Phillips et al.1985), Nitidulidae (Bartlet et al. 1993; Bartlet and James 1994), Scolytidae (Byers 1989) and Chrysomelidae (Peng and Weiss 1992).

Daily mean pheromone release (DL-1+DL-2) ranges from about 1,300 to 2,310 ng (Mayhew 1994, Bashir 2000), but quantities of pheromone produced varied about 10-fold among individual beetles (Bashir et al. 2003b). Pheromone release rate by *R. dominica* was similar during the photophase (08.00-16.00 hr) but increased significantly during the scotophase (16.00-20.00 hr) (Bashir 2000), which coincided with a period of greatest flight activity (Wright and Morton 1995, Sinclair and Haddrell 1985).

Mayhew (1994) suggested that pheromone production in *R. dominica* increase with age, reaches a plateau at about three weeks, and thereafter declines. In that study, Mayhew (1994) collected pheromones from single males (about a day old) over the course of four weeks. Detailed study on longevity of adult *R. dominica* (Edde unpublished data) showed that the beetle can survive up to 54 weeks at 29-30^{0}C and 65% RH when fed *ad libitum* on wheat. Thus, Mayhew's (1994) conclusions based on beetles that are four weeks old may not really reflect the pheromone dynamics of *R. dominica*. Quantification of pheromone release production and release rate during the entire colonization period (i.e. entire lifetime of the insect) would, undoubtedly contribute to further understanding of the pheromone dynamics of *R. dominica*.

Host Finding Behavior of *R. dominica*

Primary Attraction

Host plant selection in phytophagous insects have been documented to involve two search patterns, broadly referred to as random and directed searching (Prokopy, 1986, Schoonhoven et al. 1998). Upon location of a suitable host, sustained feeding and oviposition (i.e. acceptance) may depend on appropriateness of the plant in terms of its

quality (Jermy et al. 1988, Schoonhoven et al. 1998). Unlike random searching where the insect literally 'bumps' into potential food sources, directed searching involves orientation to the food using volatiles emitted by the plant alone or in combination with semiochemicals. Olfaction has been suggested as the most important cue utilized by most phytophagous insects during directed searching (Dicke 2000, Finch and Collier 2000), especially for *R. dominica* (Crombie, 1941). However, the question on how *R. dominica* utilizes chemical signal emitted by plants during its host finding phase has not been satisfactorily answered. This is pertinent in view of the fact that unlike other internal-feeding stored grain insect pests such as *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), which will commonly infest drying crops in the field prior to harvest (Rees 2004), *R. dominica* rarely infests crops in the field prior to harvest. The occurrence of *R. dominica* on grain, in supposedly clean stores, a few months after storage may be attributed to possible migration form natural habitats. *R. dominica* is a strong flier (Winterbottom 1922, Cotton 1950), and trapping and laboratory studies have demonstrated the species' ability to respond to host volatiles (Barrer 1983, Dowdy et. al. 1993, Mayhew 1994, Bashir 2000).

Most studies on the role of primary attraction in host plant location in *R. dominica* have been investigated using wheat and maize (Cogburn et al. 1984, Dowdy et. al. 1993, Mayhew 1994), and more recently groundnut (Bashir et al. 2001). In addition, these studies were conducted employing short-range (walking) bioassay under laboratory conditions, hence they tell us nothing on how the beetles might respond in flight. In a previous field investigation on odor-based host finding behavior of *R. dominica*, it was shown that volatiles emitted from bulk storage of wheat contributed significantly to

attraction of flying *R. dominica* (Barrer 1983). In that experiment, Barrer (1983) used between 13,000 and 15,000 tons of wheat as a source of attractant in each study site; however, one cannot be certain that male-produced pheromone sources were effectively kept out of grain bulks. Information on responses of wild *R. dominica* to aggregation pheromones released by male signaling on different plant species is lacking. The observed ability of *R. dominica* to respond to non-host volatiles (Bashir et al. 2001), and to survive on diverse plant species (Potter 1935, Linsely 1944, Wright et al. 1990) underscores the need for further studies on a wider range of host plants to establish the factors influencing primary attraction to host plant location in *R. dominica*, and may be useful in providing models on how the species can readily complete the entire host plant finding sequence in the field.

Secondary Attraction

Upon locating food sources, male *R. dominica* release two aggregation pheromones, Dominicalure-1 (DL-1) and Dominicalure-2 (DL-2), which augment recruitment of conspecifics to located food resources. The nature and biological characteristics of *R. dominica* aggregation pheromones have been reviewed above. Similar to other insect species that utilize aggregation pheromones (Fadamiro et al. 1998, Borgemeister et al. 1999, Byers 1995, Pureswaran and Borden 2005), *R. dominica* exhibits little to no attraction to host plant volatiles under field conditions, suggesting that only a small proportion of dispersing beetles, if any, would need to employ primary attraction for host finding. Thus, secondary attraction by male-produced aggregation may be the most important factor in the host selection process. Trapping studies using

synthetic DL-1 or DL-2 or their mixture have shown that individual pheromones or their mixtures are equally attractive to both sexes, but attractancy increased with dosage (Burkholder and Ma 1985). This is in contrast to observations on the related species *P. truncatus* where Trunc-call-2 is the major attractant, and Trunc-call-1 by itself attracted few beetles (Leos-Martinez et al. 1995).

Studies by Phillips et al. (1993), Trematerra and Girgenti (1994), Likhayo and Hodges (2000) have demonstrated the synergistic effect resulting from combinations of aggregation pheromone of *Sitophilus* spp with food/host volatile on trap captures under laboratory and field conditions. Dowdy et al. (1993) suggested a synergistic effect when wheat volatiles are combined with pheromone components of *R. dominica*, but provided no data to support their claim. Mayhew (1994) showed that a combination of wheat volatiles and synthetic pheromone in a laboratory walking bioassay elicited greater response by *R. dominica* than to synthetic components of the pheromone. However, this study utilized only one component (DL-1) of the aggregation pheromones; thus, it did not address the equally important questions of how the beetle would react to the second component (DL-2) of the aggregation pheromone or mixtures of DL-1 and DL-2 when combined with wheat or other host volatiles. Similar studies by Bashir (2000) indicated that both sexes of *R. dominica* more responded strongly to volatiles from male-infested wheat grains than to wheat grains alone, and that attraction was higher when thirty males were placed on 125g of wheat (supposedly higher pheromone concentration) than when the same quantity of grain was infested with only five males (a lower pheromone concentration), or to uninfested wheat grains alone. The methodology adopted by Bashir (2000), however, makes it impractical to ascertain true synergism as it is not feasible to

delineate responses induced by aggregation pheromones or plant volatiles alone or by combined action of host volatiles and aggregation pheromone.

Aggregation pheromones of phytophagous insects, including stored-product insects, have been used in monitoring, mass trapping and attracticide as components of integrated pest management strategies. Successful use of these technologies will depend largely on an understanding of insect behavior and on lure efficiency.

Research Approach

The approach adopted in this study was to elucidate the chemical aspects of habitat affinities and pheromone-mediated host selection behavior of *R. dominica*, which may be divided into two broad categories. The first part of the dissertation investigated flight activity of the insect in different habitats using synthetic pheromones. Because accurate characterization of the flight activity of an insect depends on effective monitoring techniques, preliminary studies were conducted to investigate factors such as trapping height and trap designs on efficiency of trapping *R. dominica* in different habitats. An attempt was made to characterize seasonal flight activity of the insect near grain elevators and in forest habitats. The present study is the first attempt to investigate the effects of habitat and climatic factors on fluctuation of *R. dominica* densities in central Oklahoma. A flight index model for predicting *R. dominica* was developed based in part on findings from the present study. The second part of the study examines reproductive success of *R. dominica* on different plant species, and investigated the influence of adult diet on pheromone production by *R. dominica.* A unique feature of this study is that it represents the first attempt to use diverse plant species (grain and non-grain) as food sources to

investigate nutritional ecology of *R. dominica* in relation to the influence of host plant on host location. This work also documents the first interspecific response by non-coleopteran species to *R. dominica* pheromones. Specifically, the study was addressed through the following five main objectives:

I. Evaluate factors that enhance consistency and efficiency of a trapping program for *R. dominica*. Some of the factors evaluated were trap design, trap height, and habitat.

II. Characterize *R. dominica* seasonal abundance and flight activity patterns near grain storage facilities and in forest sites in central Oklahoma, and develop models that could be used to predict *R. dominica* flight activity patterns in different habitats in Central Oklahoma.

III. Investigate if *R. dominica* is able to orient to odors as cues at close and long range to select plant species potentially suitable for pheromone or progeny production.

IV. Investigate the influence of host plant species on reproductive success, pheromone production and responses by conspecific *R. dominica*.

List of papers in this dissertation

Below is a list of papers that were prepared, or in the process of being prepared, for publication in a journal. Each paper represents a separate chapter of this dissertation.

Edde, P. A., T. W. Phillips, and M. D. Toews. 2005. Responses of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromones as influenced by trap design, trap height and habitat. Environ. Entomol. (Accepted).

Edde, P. A., and T. W. Phillips. 2005. Field responses of non-target species to semiochemicals of stored-product Bostrichidae. Ann. Entomol. Soc. Am. (Accepted).

Edde, P. A., T. W. Phillips, C. Nansen, and M. E. Payton. 2005. Flight activity of the lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), in relation to weather. Environ. Entomol. (Submitted).

Edde, P. A., and T. W. Phillips. 2005. Host effects on reproductive success and host finding behavior of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). For Entomol. Exp. Appli. (Pre-submission review).

Edde, P. A., and T. W. Phillips, J. B. Robertson, and J. W. Dillwith . 2005. Pheromone release by male *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in the laboratory was affected by host plant, but not by beetle size. For J. Chem. Ecol. (Pre-submission review).

References

Aidoo, K. E. 1993. Post-harvest storage and preservation of tropical crops. Int. Biodeterior. Biodegrad. **32:**161-173.

Anderson, K., H. Bahn, and R. Noyes. 1995. Grain storability: an overview, pp. 9-12. *In* V. Krischik, G. Cuperus and D. Galliart (eds.), Stored product management. Oklahoma State University, Coop. Exten. Serv. Circ. E-912.

Arthur, F. H. 1996. Grain protectant: current status and prospect for the future use. J. Stored Prod. Res. 12: 293-302.

Aslam, M., D. W. Hagstrum, and B. A. Dover. 1994. The effect of photoperiod on the flight activity and biology of *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J. Kansas Entomol. Soc. 67: 107-115.

Back, E. A., and **R. T. Cotton. 1922.** Stored grain pest. USDA Bulletin 1260: 46.

Barrer, P. M. 1983. A field demonstration of odor-based finding behaviour in several species of stored grain insects. J. Stored Prod. Res. 16: 105-110.

Bartelt, R. J., and D. G. Carlson, R. S. Vetter, and T. C. Barker. 1993. Male produced aggregation pheromone of *Carpophilus mutilatus* (Coleoptera: Nitidulidae) J. Chem. Ecol. 20: 3207-3219.

Bartelt, R.J., and D. G. James. 1994. Aggregation pheromone of Australian sap beetle, *Carpophilus davidsoni* (Coleoptera: Nitidulidae). J. Chem. Ecol. 20: 3207-3219.

Barkawi, L. S., W. Francke, G. J. Blomquist, and S. J. Seybold. 2003. Frontalin: De novo biosynthesis of an aggregation pheromone component by *Dendroctonus* spp. Bark beetles (Coleoptera: Scolytidae). Insect Biochem. Mol. Bio. 33: 773-788.

Bashir, T. 2000. Pheromone communication and host finding behavior of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Ph.D. dissertation, University of Greenwich, U.K.

Bashir, T., L. A. Birkinshaw, D. R. Hall, and **R. J. Hodges. 2001.** Host odours enhance the responses of adult *Rhyzopertha dominica* to male-produced aggregation pheromone. Entomol. Exp. et Appli. 101: 273-280.

Bashir, T., R. J. Hodges, L. A. Birkinshaw, D. R. Hall, and D. I. Farman. 2003a. Phenotypic plasticity of *Rhyzopertha dominica* pheromone signaling: The effects of different hosts and presence of conspecific females on male produced aggregation pheromone. J. Chem. Ecol. 29: 945-959.

Bashir, T., L. A. Birkinshaw, D. Farmn, D. R. Hall, and R. J. Hodges. 2003b. Pheromone release by *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in the laboratory: daily rhythm, inter-male variation and association with body weight and/or boring activity. J. Stored Prod. Res. 39: 159-169.

Benhalima, H, M. Q. Chaudhry, K. A. Mills, and N. R. Price. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J. Stored Prod. Res. 40: 241-249.

Birkinshaw, L. A. 1998. Mate choice in *Prostephanus truncatus* (Coleoptera: Bostrichidae): The role of male-produced aggregation pheromones. PhD. dissertation. University of Leicester, Leicester, UK.

Borgemeister, C., K. Schaefer, G. Goergen, S. Awande, M. Setamou, and H. -M. Poehling. 1999. Host-finding behavior of *Dinoderus bifoveolatus* (Coleoptera:

Bostrichidae), an important pest of stored cassava: The role of plant volatiles and odors of conspecifics. Ann. Entomol. Soc. Am. 92: 766-771.

Burkholder, W.E and M. Ma. 1985. Pheromones for Monitoring and Control of Stored-Product Insect. Ann. Rev. Entomol. 30: 257-272.

Butenandt, von A., R. Beckmann, D. Stamm, and E. Hecker, E. 1959. Über den sexuallockstoff des seidenspinners *Bombyx mori*. Reindarstellung und konstitution. Z. Naturforsch. B. 14:283-284.

Byers, J. A. **1989**. Chemical ecology of bark beetles. Experientia. 45: 271-283.

Byers, J. A. **1995**. Host tree chemistry affecting colonization in bark beetles, pp 154-213. *In* R. T. Cardé, and W. J. Bell (eds.), Chemical ecology of insect 2. Chapman and Hall, New York. NY.

Causton, C. E., and P. A. Rangel. 2002. Field observation on the biology and behavior of *Dasiops caustonae* Norrbom and McAlpine (Diptera: Lonchaeidae), as a candidate biocontrol agent of *Passsiflora mollissima* in Hawaii. J. Appl. Entomol. 126: 169-174.

Chittenden, F. H. 1911. The lesser grain borer and the larger grain borer. Bull. U.S. Bur. Entomol. 96: 29-47.

Cline, D. L. 1973. Rearing the lesser grain borer on ground dog biscuit to obtain mature larvae and pupae. Ann. Entomol. Soc. Am. 66: 551-552**.**

Cogburn, R. R., W. E. Burkholder, and H. J. Williams. 1984. Field test with the aggregation pheromone of the lesser grain borer (Coleoptera: Bostrichidae). Environ. Entomol. 13: 162-166.

Cork, A., D. R. Hall, R. J. Hodges, and J. A. Pickett. 1991. Identification of major components of the male produced aggregation pheromones of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). J. Chem. Ecol. 17: 789-803.

Cotton, R. T. 1950. Insect pests of stored grain and grain products. Burgess Publishing Company. Minneapolis, Minnesota.

Crombie, A. C. 1941. On oviposition, olfactory conditioning and host selection in *Rhyzopertha dominica* Fab. (Insecta, Coleoptera). J. Exp. Biol. 18: 62-79.

Dicke, M. 2000. Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. Biochem. Systematics and Ecol. 28: 601 – 617.

Doan, R. W. 1919. Weevils in Australian in California. J. Econ. Entomol. 12: 308-312.

Doolittle, R. E., J. H. Tumlinson, A. T. Proveaux, and R. R. Heath. 1980. Synthesis of the sex pheromone of the Japanese beetle. J. Chem. Ecol. 6:473-485.

Dowdy, A. K. 1994. Flight initiation of lesser grain borer (Coleoptera: Bostrichidae) as influenced by temperature, humidity and light. J. Econ. Entomol. 1714-1717.

Dowdy, A. K., R. W Howard, L. M. Seitz, and W. E. McGaughey. 1993. Responses of *R. dominica* (Coleoptera: Bostrichidae) to its aggregation pheromone and wheat volatiles. Environ. Entomol. 22: 965-970.

Dzhumakulov, T., and M. M. Kadyrova. 1992. Synthesis of hexadec-9Z-enal - one of the components of the sex pheromone of *Heliothis armiger*a. Chem. Nat. Comp. 28:240-241.

Edde, P. A., and T. W. Phillips. 2005a. Field responses of non-target species to semiochemicals of stored-product Bostrichidae. Ann. Entomol. Soc. Am. (in press)**.**

Edde, P. A., T. W. Phillips, and M. D. Toews. 2005b. Responses of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromones as influenced by trap design, trap height and habitat. Environ. Entomol. (in press).

Elkinton, J.S., D. I. Wood, and L. B. Hendry. 1980. Pheromone production by the bark beetle, *Ips paraconfusus*, in the non-host, white fir. J. Chem. Ecol. 6: 979-987.

Fadamiro, H. Y., I. Gudrups, and R. I. Hodges. 1998. Upwind flight of *Prostephanus truncatus* is mediated by aggregation pheromone but not food volatiles. J. Stored Prod. Res. 34: 151-158.

Federal Grain Inspection Service (FGIS). 1997. Wheat. In Grain inspection handbook, Book II, grain grading procedure, USDA-GIPSA-FIGS, Washington, DC.

Finch, S. and **R. H. Collier. 2000.** Host-plant selection by insects- a theory based on 'appropriate/inappropriate landing' by pest insects of cruciferous plants. Entomol. Exp. et Appli. 96. 91-102.

Fisher, W. S. 1950. A revision of the North America species of beetles belonging to the family Bostrichidae. Miscellaneous Publication No 698. USDA, Washington, DC.

Flinn, P. W., D. W. Hagstrum, and T. W. Phillips. 2004. Stimulation model for *Rhyzopertha dominica* population dynamics in concrete grain bins. J. of Stored Prod. Res. 40: 39-45.

Ghorpade, K. D., and K. S. Thyagarajan. 1980. A reliable character for sexing live or dead *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). J. Stored Prod. Res. 16: 151-153.

Gorham, J. R . 1987. Insects and mites in food: an illustrated key. Agricultural Handbook Number 655. U.S. Department of Agriculture, Washington, DC.

Hagstrum, D. W. and P. W. Flinn. 1994. Survival of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in stored wheat under fall and winter temperature conditions. Environ. Entomol. 23: 390-395.

Haines, C. P. (ed.). 1991. Insect and Arachnid of Tropical Stored Products: their biology and identification. Natural Resources Institute, 2nd edition. Natural Resources Institute, Chatham, UK.

Hagstrum, D. W., C. Reed, and P. Kenkel. 1999. Management of stored wheat insect pests in the USA. Integrated Pest Man. Rev. 4: 127-142.

Harein, P. and R. Meronuck. 1995. Stored grain losses due to insects and molds and the importance of proper grain management, pp. 29-31. *In* V. Krischik, G. Cuperus and D. Galliart (eds.), Stored product management. Oklahoma State University, Coop. Exten. Serv. Circ. E-912.

Helms, S. E., and M. D. Hunter. 2005. Variation in plant quality and the population dynamics of herbivores: there is nothing average about aphids. Oecologia 145: 196-203.

Hodges, R. J., A. Cork, and D. R. Hall. 1984. Aggregation pheromones for monitoring the greater grain borer *Prostephanus truncatus*, pp. 255-260. *In* Proceedings British Crop Protection Conference. Pest and Diseases. November 1984, Brighton, UK.

Howe, R. W. 1950. The development of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) under constant temperature. Entomol. Monthly Mag. 86: 1-5.

Hughes, P. R., and J. A. A. Renwick. 1977. Hormonal and host factors stimulating pheromone synthesis in female western pine beetles *Dendroctonus brevicomis.* Physiol. Entomol. 2: 289-292.

Islam, N., R. Bacala, A. Moore, and D. Vanderwel. 1999. Biosynthesis of 4-methyl-1-nonanol: Female-produced sex pheromone of the yellow mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae). Insect Biochem. Mol. Biol. 29: 201-208.

Ivie, M. A. 2002a. Family 69. Bostrichidae, pp 233-244. *In* R. H. Arnett, Jr., M. C. Thomas, P. E. Skelley, and J. H. Frank (eds.), American beetles, Vol. 2; Polyphaga: Scarabaeoidae through Curculionoidea. CRC press, Boca Raton.

Ivie, M. A. 2002a. Keys to families of beetles in America north of Mexico, pp 816-835. *In* R. H. Arnett, Jr., M. C. Thomas, P. E. Skelley, and J. H. Frank (eds.), American beetles, Vol. 2; Polyphaga: Scarabaeoidae through Curculionoidea. CRC press, Boca Raton.

Jermy, T., A. Szentesi, and J. Horvath. 1988. Host plant finding in insects: the case of the Colorado potato beetle. Entomol. Exp. et Appli. 49. 1-21.

Karlson, P. and A. Butenandt. 1959. Pheromones (ectohormones) in insects. Ann. Rev. Entomol. 4: 39-58.

Khorramshi, A., and W. E. Burkholder. 1981. Behavior of the lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J. Chem. Ecol. 7: 33-38.

Kumar, H. 2001. Resistance in maize to the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). J. Stored Prod. Res. 38: 267-280.

Landolt, P. J., and T. W. Phillips. 1997. Host plant influence on sex pheromone behavior of phytophagous insects. Ann. Rev. of Entomol. 42: 371-391.

Langor, D. W., J. R. Spencer, and G. R. Pohl. 1990. Host effects on fecundity and reproductive success of *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). Evol. 44: 609-618.

Leal, W. S. 2001. Molecules and macromolecules involved in chemical communication of scarab beetle. Pure. Appli. Chem. 73: 613-615.

Leconte, J. L. 1862. Classification of the Coleoptera of North America. Smithsonian Misc. Collection 3: 207-208.

Leos-Martinez, J., T. A. Granovsky, H. J. Williams, S. B. Vinson, and W. E. Burkholder. 1986. Estimation of the aerial density of the lesser grain borer (Coleoptera: Bostrichidae) in a warehouse using dominicalure traps. J. Econ. Entomol. 79: 1134-1138.

Leos-Martinez, J., Socorro-Gonzalez-Alonso M.del S., and H. J. Williams. 1995. Optimization of pheromonal trapping methods for *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). J. Stored. Prod. Res. 31: 103-109.

Levinson, H. Z., A. R. Levinson, T. -L.Jen, J. L. D. Williams, G. Kahn, and W. Francke. 1978. Production site, partial composition and olfactory perception of a pheromone in the male hide beetle. Naturwissenschaften. 65:543.

Likhayo, P. W., and R. J. Hodges. 2000. Field monitoring *Sitophilus zeamais* and *Sitophilus oryzae* (Coleoptera: Curculionidae) using refuge and flight traps baited with synthetic pheromone and cracked wheat. J. Stored Prod. Res. 36: 341-353.

Linn, C.E., L. B. Boosted, J.W. Due, and W. L. Roelofs. 1984. Redundancy in a chemical signal: behavioral responses of male *Trichoplusia ni* to a 6-component sex pheromone blend. J. Chem. Ecol. 10: 1635-1658.

Linsley, G. E. 1944. Natural sources, habitat, and reservoirs of insects associated with stored food products. Hillarie 16: 187-225.

Longstaff, B. C., and N. T. Starick. 1989. The influence of media on the survival of pupae of *R. dominica* (Coleoptera: Bostrichidae) after sexing. J. Stored Prod. Res. 25: 93-95.

Lorini, I., and D. J. Galley. 1999. Delthametrin resistance in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), a pest of stored grain in Brazil. J. Stored Prod. Res. 35: 37-45.

Market, K. A., and M. A. Ivie. 2003. Beetle fauna of the United State and Canada. The Coleopterist Bulletin. 57: 495-503.

Mathew, G. 1987. Insect borers of commercially important stored timber in state of Kerala, India. J. Stored Prod. Res. 23: 185-190.

Mayer, M. S., and J. R. McLaughlin. 1991. Handbook of insect pheromone and sex attractant. CRC Press Inc. Boca Raton.

Mayhew, T. J. 1994. Pheromone production and behavioral response to pheromones in the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). MS thesis, University of Wisconsin-Madison, USA.

McBrien, H. L., J. G. Millar, R. E. Rice, J. S. McElfresh, E. Cullen, and F. G. Zalom. 2002. Sex attractant pheromone of the red-shouldered stink bug *Thyanta pallidovirens*: a pheromone blend with multiple redundant components. J. Chem. Ecol. 28:1797-818.

Moreira, J.A., and A.G. Correa. 2003. Enantioselective synthesis of three stereoisomers of 5,9-dimethylpentadecane, sex pheromone component of *Leucoptera coffeella*, from (-)-isopulegol. Tetrahedron: Asymmetry.14:3787-3795.

Morrison, A. A., P. G. Mulder, and K. Sambaraju. 2005. Host suitability of pecans for five species of storage pests. Proc. Oklahoma Pecan Growers' Assoc. 75: 55-58.

Naoshima, Y., M. Kawakubo, S. Wakabayashi, and S. Hayashi. 1981. Synthesis of (Z)-7-eicosen-11-one and (Z)-7-nonadecen-11-one, the sex pheromone of peach fruit moth. Agric. Biol. Chem. 45:439.

Nojima, S., P. S., Robbins, G. A., Salsbury, B. D. Morris, W. L. Roelofs and M. G. Villani. 2003. L-Leucine methyl ester: The female-produced sex pheromone of the scarab beetle *Phyllophaga lanceolata*. J. Chem. Ecol. 29: 2439-244.

Peng, C. W., and M. J. Weiss. 1992. Evidence of an aggregation pheromone in the flea beetle, *Phyllotreta crucifera* (Goese) (Coleoptera: Chrysomelidae). J. Appl. Entomol. 18: 875-884.

Phillips, T. W., X. L. Jiang, Burkholder, W. E., Phillip, J. J. and Q. Tran. 1993. Behavioral responses to food volatiles by two species of stored-product Coleoptera, *Sitophilus oryzae* (Curculionidae) and *Tribolium castaneum* (Tenebrionidae). J. Chem. Ecol. 19: 723-734.

Phillips, J K., C. A. Walgenbach, J. A. Klein, W. E. Burkholder, N. R. Schmuff, and H. M. Fales. 1985. (R*,S*)-5-hydroxyl-4-methyl3-heptanone male produced aggregation pheromone of *Sitophilus oryzae* (L.) and *S. zeamais* Motsch. J. Chem. Ecol. 11: 163-1274.

Potter, C. 1935. The biology and distribution of *Rhyzopertha dominica* (Fab.). Trans. Royal Entomol Soc. 83: 449-482.

Prokopy, R. J. 1986. Visual and olfactory stimulus interaction in resource finding by insects. *In* Payne, T. L., M. C. Birch, and C. E. J. Kennedy (eds.). Mechanism in Insect Olfaction. Clarendon Press. Oxford, UK

Pureswaran, D. S., and J. H. Borden. 2005. Primary attraction and kairomonal host discrimination in three species of *Dendroctonus* (Coleoptera: Scolytidae). Agric. For. Entomol. 16: 765-782.

Rees, D. 2004. Insects of stored products. CSIRO Publishing. Collingwood, Victoria, Australia.

Roelofs, W. L. 1995. Chemistry of sex attraction. Proc. Nat. Acad. Science. USA. 92: 44-49.

Rollin, P., and J.-R. Pougny. 1986. Synthesis of (6Z)-cis-9S,10R-epoxyheneicosene, a component of the ruby tiger moth pheromone. Tetrahedron. 42:3479-3490.

Sanchez-Marinez, R.I., M. Morales-Valdes, M. I. Silveira, M. O. Cortez-Rocha, and F. Ortega-Dorame. 1997. End-use quality of flour from *Rhyzopertha dominica* infested wheat. Cereal Chem. 74: 481-483**.**

Schäfer, K., G. Goergen, and C. Borgemeister. 2000. An illustrated identification key to four different species of adult *Dinoderus* (Coleoptera: Bostrichidae), commonly attacking dried cassava chips in West Africa. J. Stored Prod. Res. 36: 245-252.

Schoonhoven, L. M., T. Jermy, and J. J. A. van Loon. 1998. Insect-plant biology: from physiology to evolution. New York.

Schwardt, H. H. 1933. Life history of the lesser grain borer. J. Kansas Entomol. Soc. 2:61-66.

Silverstein, R.M., J O. Rodin, and D. L. Wood. 1966. Sex attractants in frass produced by male *Ips confusus* [now *I. paraconfusus*] in ponderosa pine. Science. 154: 509-510.

Sinclair, E. R. 1981. Sexing live adult *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Trans. Royal Entomol. Soc. London. 83: 449-482.

Sinclair, E. R., and R. L. Haddrell. 1985. Flight of stored products beetles over a grain farming area in southern Queensland. J. Aust. Entomol. Soc. 24: 9-15.

Singh, H. and J. N. Liles. 1972. Effect of gamma rays on the acute lethality and reproductive potential of lesser grain borer adults. J. Econ. Entomol. 65: 656-659.

Slansky, F. 1982. Toward nutritional ecology of insects. *In* Proceedings, 5th international symposium on insect-plant relationship. Pudoc, Wageningen.

Stemley, P. G., D. A. Wilbur. 1966. A color characteristic for sexing live lesser grain borers. J. Econ. Entomol. 59: 760-761.

Subchev, M. A. 1981. Z-11-Hexadecenyl acetate: sexual attractant for males of eight species of moths (Insecta, Lepidoptera). Ekologiya. 9:59-62.

Tanaka, Y., H. Honda, K. Ohsawa, and I. Yamamoto. 1986. A sex attractant of the yellow mealworm Tenebrio molitor L., and its role in mating behavior. J. Pesticide Science 11: 49-55.

Thompson, V. 1966. Biology of the lesser grain bore, *Rhyzopertha dominica* (F.). Bull. Grain Tech. 4:163-168.

Throne, J. E., and L. D. Cline. 1994. Seasonal flight activity and seasonal abundance of selected stored-product Coleoptera around grain storages in South Carolina. J. Agric. Entomol. 11: 321-338.

Tillman, J. A., S. J. Seybold, R. R. A. Jurenka, and G. J. Blomquist. 1999. Insect pheromone – an overview of biosynthesis and endocrine regulation. Insect Biochem. Mol. Bio. 29: 481-514.

Tolasch, T., C. Borgemeister, Y. Masuda, K. Fujita, K. Mori, and W. Francke. 2002. The aggregation pheromone of *Dinoderus bifoveolatus* Wollaston (Coleoptera: Bostrichidae). *In* Proceedings, 19th annual meeting International Society of Chemical Ecology 3-7 August 2002, University of Hamburg, Germany.

Trematerra, P., and P. Girgenti. 1994. Influence of pheromone and food attractant on trapping of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae): a new trap. J. Appl. Entomol. 108: 12-20.

Tumlinson, J. H. and P. E. A. Teal. 1987. Relationship of structure and function to biochemistry in insect pheromone system. *In* G. J. Blomquist, and G. D. Presttwich [eds.], Pheromone Biochemistry. Academic Press, Orlando, Fl.

Vanderwel, D., B. Johnston, A. C. Ocehsclager, 1992. Cucujolide biosynthesis in the merchant and rusty grain beetle. Insect. Biochem. Mol. Biol. 22: 875-883.

Vanderwel, D., and A. C. Ocehsclager, 1987. Biosynthesis of pheromone and endocrine regulation of pheromone production in Coleoptera, pp 175-215. *In* G. D. Prestwich, and G. J. Blomquist. Pheromone biochemistry. Academic Press, Orlando, Florida.

Williams, H. J., R. M. Silverstein, W. E. Burkholder, and A. Khorramshahi. 1981. Dominicalure 1 and 2: components of aggregation pheromone from male lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). J. Chem. Ecol. 7: 759-781.

Wilson, E. O. 1980. Sociobiology: The abridge edition. Belknap Press, Harvard University, Cambridge, MA.

Winterbottom, D. C. 1922. Weevil in wheat and storage of grain in bags. A record of Australian experience during the war period (1915 to 1919). South Australian Government, Australia.

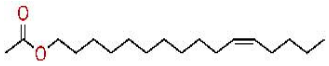
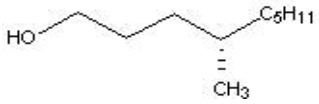
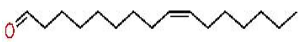
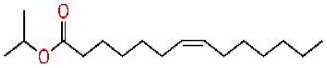
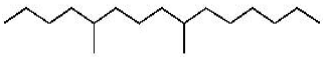
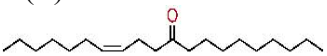
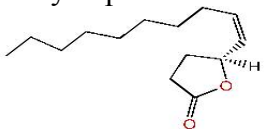
Wright, V. F., E. E. Fleming, and D. Post. 1990. Survival of *Rhyzopertha dominica* (Coleoptera, Bostrichidae) on fruits and seeds collected from woodrat nests in Kansas. J. Kans. Entomol. Soc. 63*:* 344-347.

Wright, E. J., and R. Morton. 1995. Daily flight activity of *Trogoderma variabile* (Coleoptera: Dermestidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J. Stored Prod. Res. 31: 1777-184.

Yao, M. C., and K. C. Lo. 1994. Phoxim Resistance in *Rhyzopertha dominica* Fabricius in Taiwan. Chinese J. Entomol. 14: 331-341.

Zvereva, E. L. 2002. Effects of host plant quality on overwintering success of the leaf beetle *Chrysomela lapponica* (Coleoptera: Chrysomelidae). Eur. J. Entomol. 99: 189-195.

Table 1. Example of compounds used as insect pheromone

Compound	Example	species	Reference
Acetate	(Z)-11-Hexadecenyl acetate 	*Wiltesia crataegella* Hübner	Subchev (1981)
Alcohol	4-Methyl-1-nonanol 	*Tenebrio molitor* L.	Islam et al. (1999)
Aldehyde	Z)-9-Hexadecenal 	*Heliothis armigera* (Hübner)	Dzhumakulov and Kadyrova, (1992)
Epoxide	(Z,Z)-3,6-(9S,10R)-9,10-Epoxyheneicosadiene 	*Phragmatobia fuliginosa* L.	Rollin and Pougny (1986)
Esters other than acetate	Isopropyl (Z)-7-tetradecenoate 	*Dermestes maculatus* De Geer	Levinson et al. (1978)
Hydrocarbon	5,9-Dimethylpentadecane 	*Leucoptera coffeella* (Guérin-Mèneville)	Moreira and Correa (2003)
Ketone	(Z)-7-Eicosen-11-one 	*Carposina niponensis* Walsingham	Naoshima et al. (1981)
Lactone	(R,Z)-5-(Dec-1-enyl)-oxacyclopentan-2-one 	*Popillia japonica* Newman	Doolittle et al. (1980)

CHAPTER II

RESPONSES OF *RHYZOPERTHA DOMINICA* (COLEOPTERA: BOSTRICHIDAE) TO ITS AGGREGATION PHEROMONES AS INFLUENCED BY TRAP DESIGN, TRAP HEIGHT AND HABITAT.

Accepted for publication in Environmental Entomology on 9 August 2005

Environmental Entomology

Subject matter: Population Ecology

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Responses of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) to its Aggregation Pheromones as Influenced by Trap Design, Trap Height and Habitat

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Abstract

Lindgren multiple funnel traps and Japanese beetle traps captured more lesser grain borers, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), than did Pherocon II sticky traps or bucket traps when all were baited with the same aggregation pheromones. Bucket traps captured six-fold fewer beetles than Lindgren four-unit traps. Retentions of captured *R. dominica* were not significantly different in traps with soapy water or dry insecticide as killing agents for either trap design, but were significantly higher than those retained in traps lacking a killing agent. Lindgren eight-unit funnel traps captured a similar number of *R. dominica* when compared with the four-unit funnel traps. More beetles were captured near grain storage facilities than in forests or in open fields. Trap height (1, 2 or 4 m above the ground) had no detectable effect across habitat, but significantly interacted with habitat. Traps placed 1 or 2 m high near grain elevators and in open fields captured similar numbers of beetles, and yielded higher catches of *R. dominica* than traps placed 4 m high in these habitats. The reverse was true in forest habitats. Captured *R. dominica* were similarly female-biased in all trap designs, and the proportion of females to males did not differ among trap heights or habitats in which they were trapped.

Keywords: Stored-product insects, lesser grain borer, pheromones.

Introduction

THE LESSER GRAIN BORER, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is a serious pest of stored grain worldwide. Life history and development of *R. dominica* was described in early work (e.g., Potter 1935; Crombie 1941). *R. dominica* is highly polyphagous and has been recorded feeding on diverse food crops such as legumes, tubers, bulbs, cereals and packaging material made from wood (Potter 1935). Susceptible cereal crops include wheat, maize, rice and sorghum (Hagstrum et al. 1999). *R. dominica* infested-grain decreases in value as a function of live insects, insect damaged kernels, or insect fragments in milled products. Unlike most primary stored-grain pests, *R. dominica* is not known to attack cereals in the field, but it is a strong flier and has been found infesting grain, in supposedly clean stores, within weeks or months after storage (Gates 1995). This rapid colonization behavior, strong flight ability and broad polyphagy, coupled with the fact that *R. dominica* has been trapped in diverse environments, including woodlands substantial distances from grain stores (Cogburn 1988), led us to suspect movement of this pest between potentially natural habitats and grain storage facilities.

Current management strategies for *R. dominica* involve the use of broad-spectrum insecticides, particularly organophosphates or pyrethroid grain protectants such as malathion, pirimiphos-methyl, chlorpyrifos-methyl, and deltamethrin (Arthur 1996). Insecticides are effective in many cases, but insecticide resistance is evident in many populations of *R. dominica,* due to excessive use (Benhalima et al. 2004). Additionally, insecticides can be harmful to nontarget species and may pollute the environment **(**Lorini

and Galley 1999). Thus, there is a need to develop sustainable and environmentally friendly pest management tactics.

Effective control of *R. dominica,* as well as other stored-grain pests, with minimal insecticide use requires an integrated management approach combining sanitation, monitoring, and other preventive practices, including use of pheromone-baited traps. Pheromone traps can detect pests, monitor their distributions in storage facilities, and possibly manipulate their populations (Phillips et al. 2000). The two male produced aggregation pheromones of *R. dominica* are (S)-(+)-1-methylbutyl (*E*)-2-methyl-2-pentenoate (dominicalure-1 or DL-1) and (S)-(+)-1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate (dominicalure-2 or DL-2) (Williams et al. 1981). Both pheromones are equally attractive to both sexes of the beetle in the field and in the laboratory. Sensitive and reliable pheromone-baited traps are needed for *R. dominica*.

Factors that may affect efficacy of pheromone-baited traps include: target species, trap design, trap height, time of day, dosage of pheromone per trap, and habitat **(**Barak et al. 1991; de Groot and DeBarr 1998**;** Boucher et al. 2001). Consideration of these factors would enhance consistency and efficiency of a trapping program for *R. dominica*. Our objectives, therefore, were to evaluate the effects of trap design, trap height, and habitat on capture of *R. dominica* in pheromone-baited traps.

Materials and Methods

Pheromone lures. Pheromone lures used in the experiments were fabricated in our laboratory. Briefly, number 11.5 sleeve stoppers (Fisher Scientific, Pittsburgh, PA; referred to here as rubber septa) were first cleaned by soaking overnight in

dichloromethane and then allowed to air dry under a fume hood for 24 h. Pheromones were applied to the interior of a rubber septum via a 50% hexane solution containing 5 mg each of DL-1, DL-2. Treated rubber septa were affixed to traps as described below, and new lures were used for each trapping period in each experiment**.**

Experimental Series 1: Trap Comparisons. Four trap designs for flying insects were evaluated in 2000 in an open field near Stillwater, OK (Open Field I, Table 1). The study site was selected based on: proximity to our laboratory, sustained high *R. dominica* population, and a large plot size to accommodate the large number of traps in the experiment. Trap types evaluated in the first study were: the Lindgren four-unit multiple funnel traps (PheroTech Inc., British Columbia, Canada), Japanese beetle traps (Trécé Inc., Salinas, CA), Pherocon II traps (Trécé Inc., Salinas, CA), and Unitrap or bucket traps (Agrisense/Biosys, Columbia, MD) (Fig. 1). Lindgren funnel traps consist of a series of vertically connected black-plastic cone-shaped funnels terminating in a white-plastic collection jar at the bottom (Lindgren 1983). A pheromone lure was suspended by a wire at the mid-point inside the second funnel from the bottom of each trap. Japanese beetle traps have four-finned (omnidirectional) veins on the top of a tapered cone leading to a collection cup. Japanese beetle traps used in this study were yellow. A pheromone lure was affixed by a wire to the center of one fin on each Japanese beetle trap. Pherocon II traps were diamond-shaped white cardboard traps designed to capture flying insects such as moths and beetles on an inner sticky surface. A pheromone lure was simply placed on the center of the bottom sticky surface of each pherocon II trap. Bucket traps consist of a funnel-shaped plastic receptacle with a lid and holder for attaching lures, mounted over a bucket for retaining captured insects. A pheromone lure was suspended

within the funnel, attached at the lid, of each bucket trap. Bucket traps used in the study had white colored receptacles and green lids. Modifications to traps included inserting a finer screen in the collection cups of the Lindgren traps and replacing the collection cup of the Japanese beetle traps with a 100 ml glass jar. These modifications were necessary because *R. dominica* could escape through the original equipment. With the exception of Pherocon II traps that had a sticky surface, captured insects were prevented from escaping by placing pieces of No-Pest® Strip (United industries Corp., St. Louis, MO; active ingredient: dichlorvos) in trap receptacles. Traps were hung from vertical polyvinyl chloride pipe stands we inserted into the soil, about 1.7 m above the ground, which placed traps above the grass that generally grew to a height of ~1 m. The first study was conducted as a randomized complete block design from 30 May to 15 June 2000. Eight experimental blocks of traps were deployed in the field as separate trap lines in east-west orientations, perpendicular to the prevailing southerly winds, in which each of the four trap types was represented once and randomly assigned a position in each block. There were 15-20 m between traps in a block and at least 50 m between blocks.

A second experiment was conducted in the same field to compare the efficacy of Lindgren four-unit traps versus the longer eight-unit funnel traps of the same basic design in capturing *R. dominica*. The experiment was conducted from 16 August to 1 September 2000 and was deployed as a completely randomized design in which four traps of each of the two designs were randomly arranged in the field with a minimum of 80 m between traps.

Experimental Series 2: Effect of trap height and habitat on capture of *R. dominica*: We tested the hypothesis that captures of *R. dominica* in pheromone-baited traps would vary due to differences in trap height and habitat, which may reflect optimal flight height an habitat preference of *R. dominica*, by conducting an experiment from 7 July to 10 October 2002 at six locations (Table 1). Two of each of three different habitats was used: a forest, an open agricultural field, and an open field adjacent to a grain storage facility, hereafter referred to as wooded, open field and grain elevator, respectively. Based on the results of Experimental Series 1, the Lindgren four-unit multiple funnel traps were used in this experiment. Three traps baited with pheromone lures were deployed at each of the six locations, and each trap was assigned to one of three heights at each location. Trap heights were measured as the distance from the ground to the bottoms of the collection cups: 1 m, 2 m, or 4 m,. The 1 and 2 m high traps were hung from vertical polyvinyl chloride pipe stands equipped with a horizontal top arm. Those at 4 m were attached to ropes hung on the top vertical arms of 5 m vertical metal pipes inserted in the ground. Ropes where run through pulleys bolted to the arm of the pipe to facilitate trap servicing. The three traps at each location were spaced 15- 20 m apart and arranged in an east-west orientation, perpendicular to the prevailing southerly winds. Traps at the grain elevator sites were placed at least 6 m away from grain bins. Soapy water was used in the collection cups to prevent captured insects from escaping. Trap positions were rotated weekly at each location to minimize positional effect on trap catch. The study was organized as a two-factor experiment. The main factors in the experiment were habitat types and trap height, each of which had three levels, and there were two replicates represented by the two habitat locations of each type. Trapping occurred at each location

for a one-week period and was repeated for fifteen weeks, so that 30 replications were accumulated.

Experimental Series 3: Retention of Trapped Insects. *R. dominica* captured in our trap comparison study and in the habitat and trap height study were restrained with insecticide strips and soapy water, respectively. However, it has been suggested that different trap designs, supplied with different killing agents, might be differentially effective in retaining captured insects (Morewood et al. 2002; de Groot and Nott 2003). We therefore investigated retention of captured *R. dominica* in pheromone-baited Lindgren four-unit funnel and Japanese beetle traps using different killing agents. We choose the Lindgren four-unit funnel and Japanese beetle traps because results from Experimental Series 1 suggested that these two trap types were equally effective in capturing *R. dominica*, and these captured *more R. dominica* than others did.

The killing agents tested were soapy water and insecticide strips emitting dichlorvos. About 60 ml of soapy water (2% v/v of Palmolive® washing liquid soap, Colgate-Palmolive Company, New York, NY) was added to the collection cups of both trap designs, hereafter referred to as wet traps. Dichlorvos was gradually emitted from a 2 x 3 x 0.8 cm blocks cut from a No-Pest® strip placed in the collection cups of the tested trap designs, hereafter referred to as dry traps. Control traps consisted of pheromone baited Lindgren funnel traps and Japanese beetle traps left blank i.e. with neither soapy water nor dichlorvos. Lindgren funnel traps used for wet trapping were further modified by placing 100 ml plastic cups in the collection cups to prevent drainage of soapy water through the wire mesh in bottom of the cups. The modified Japanese beetle trap had no

drainage holes, however, no rainfall occurred for the six-day duration of the experiment (Stillwater weather data: http://www.mesonet.org).

The bioassay was conducted from 6 September to 12 September 2004 in forest habitats (Table 1). Based on the results from Experimental Series 2, the forest habitat was selected for the retention study. The traps, arranged in east-west orientations, were hung from PVC pipes about 2 m above ground, and were spaced 15-20 m apart. The experimental design was a completely randomized block in which each of the two forest sites represented a block. Treatments were replicated three times in each block to yield six replications.

Sex Ratio. Sexes were determined from sampled insects in Experimental Series 1 and 2 in order to determine if different trap designs, trap height and habitats affected sex ratio of captured *R. dominica*. Sex was determined by squeezing the abdominal body region to extrude their genitalia, which were viewed under a dissecting microscope (Crombie 1941). Generally, 30% or more of the insects captured in each trap was sexed, but this number differed based on insect condition. Insects in the Experimental Series 2 were sampled from all treatments at five different trap-check dates. Because the numbers of beetles sexed were not equal between treatments within experiments, data were standardized by converting the number of males and females sexed per treatment into proportion of the number of beetles sexed per treatment. Data on the proportions of males and female captured in Experimental Series 1 were analyzed as a two-factor experiment in which the main factors were trap design and beetle sex. Similarly, data on the proportions of males and female captured in Experimental Series 2 were analyzed as a

three-factor experiment in which the main factors were habitat type, trap height and beetle sex.

Data Analysis. Trap catch data were analyzed using SAS PROC MIXED (SAS Institute, 2001). Blocks (trap design study) and locations and weeks (habitat, trap height study) were considered as random effects in the respective mixed models and therefore included in the RANDOM statement within the PROC MIXED code. Prior to data analysis, count and percentage data were transformed using the Log(X +1) and square-root arcsine transformation methods (Zar 1999), respectively, in order to satisfy the assumptions of normality and homogeneity of variance. Actual means and standard errors are presented in the text, tables, and figures. Tukey's Studentized range test was used to separate means (Tukey 1953).

Results

Experimental Series 1: Trap design. The number of *R. dominica* captured differed significantly ($F = 32.6$; df = 3, 28; $P < 0.001$) among trap types. Lindgren four-unit funnel traps and Japanese beetle traps captured the most beetles (Fig. 2). Bucket traps captured six-fold fewer beetles than Lindgren traps. Analyses of proportions of females only revealed no significant differences among trap types ($F = 1.7$; df = 3, 28; $P = 0.193$); a similar finding was obtained when examining proportions of males captured ($F = 1.8$; df = 3, 28; $P = 0.17$). Female to male ratios were significantly female biased ($F = 100.5$; df = 1, 56; $P < 0.001$). This ratio ranged from 0.67 ± 0.08 to 0.78 ± 0.04, but did not differ among trap types ($F = 0.03$; df = 3, 56; $P = 0.992$). Capture of *R. dominica* in

Lindgren 4-funnel and 8-funnel traps was similar ($F = 0.8$; df = 1, 7; $P = 0.391$) with average captures of 55.8 ± 8.4 and 59.6 ± 4.8, respectively.

Experimental Series 2: Trap height and habitat. There was a significant interaction between habitat and trap height ($F = 5.7$; df = 4, 225; $P < 0.001$). Traps placed 1 or 2 m high near grain elevators captured more beetles than the other habitat and trap height combinations (Fig. 3). The next largest capture was in traps placed at 4 m high near grain elevators and in the forest and field sites. Mean numbers of beetles captured were not significantly different between forest and open fields when traps were placed at 1 or 2 m high. Traps at 4 m high in open fields captured the fewest *R. dominica* (Fig. 3). There was no significant main effect for the trap height factor ($F = 2.1$; df = 2, 225; $P = 0.120$); but the main effect for habitat factor was significant ($F = 64.0$; df = 2, 225; $P < 0.001$). Traps in areas adjacent to grain elevators significantly ($F = 27.5$; df = 2, 135; $P < 0.001$) (Fig. 4) captured more *R. dominica* than those in forest or open fields. The open-field habitat yielded the fewest beetles, four fold fewer than near the grain elevator sites and about half the number captured in the wooded sites (Fig. 4).

Analysis of proportion of *R. dominica* sexes captured using a three factor ANOVA test showed minimum interactions among habitat, height and sex ($F = 2.3$; df = 4, 90; $P = 0.065$). Interactive effects between trap height and beetle sex ($F = 1.3$; df = 2, 90; $P = 0.267$), and for habitat and trap height combinations ($F = 0.01$; df = 4, 90; $P = 0.999$) were not significant. However, there were significant interactions between habitat and sex ($F = 3.3$; df = 2, 90; $P < 0.04$). More females than males were captured within each habitat, but the sex ratio was consistent within habitats (Fig. 5). Trap height and

habitat were not significant in the model. Female to male ratios were significantly female biased ($F = 202.2$; df = 1, 90; $P < 0.001$) with a mean ratio of about 3:2.

Retention of captured *R. dominica* did not differ significantly between traps with soapy waters and those with dichlorvos for either trap design (Fig. 6), but these were significantly higher than those retained in control traps ($F = 7.2$; df = 5, 25; $P < 0.001$).

Discussion

Trap design significantly affected outdoor trapping of *R. dominica*. Lindgren four-unit funnel traps and Japanese beetle traps were the most effective traps for *R. dominica*. The bucket trap was least effective. It is possible that observed differences in captures of *R. dominica* were due to differences in the size of trap openings. Trap openings refer to exposed portions of the traps through which beetles gain unhindered access into traps. This is approximately 1194.5, 810.5, 450.0 and 235.7 cm^2 in Lindgren four-unit traps, Japanese beetle traps, Pherocon II traps and bucket traps, respectively. Traps with larger openings, such as Lindgren multiple funnel traps and Japanese beetle traps were likely easier for *R. dominica* to access and enter. Alternatively, the shapes of Lindgren multiple funnel traps and Japanese beetle traps, which may mimic the silhouette of a vertical tree trunk, could provide visual stimuli that work in concert with the chemical stimuli to elicit beetle response. A tree-like shape might have provided *R. dominica*, which is from a family of wood boring beetles, with a cue for orientation that is lacking in the other trap designs. Other species of wood-boring beetles are known to respond to tree-like traps (Borden et al. 1986; Flechtmann et al. 2000).

Although Lindgren eight-unit traps have twice as many identical openings as the four-unit funnel traps, mean capture of *R. dominica* was not significantly different between the two trap designs; this indicates that no significant improvement in trapping efficacy may be achieved beyond the optimum trap openings required to maximize access by insects into the trap. Our observations contrast with those reported for *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), in which trap capture of the beetles was doubled by doubling the heights of conventional Lindgren multiple funnel traps (Borden et al. 1986). The difference in our observations and those of Borden et al. (1986) underscore the existence of species-specificity in attractiveness of silhouettes of different lengths (i.e. some prefer longer traps, others prefer shorter traps). Lindgren eight-unit funnel traps are bulkier and more expensive than the four-unit traps; therefore, it might be more economical to employ the later or Japanese beetle traps for outdoor trapping of *R. dominica*.

Results from Experimental Series 3 confirmed that *R. dominica* responded equally to Lindgren four-unit funnel traps and Japanese beetle traps. Similarly, traps with dichlorvos pieces or traps with soapy water, for both trap designs, were equally effective in retaining captured *R. dominica*; about half as many insects were retained in traps lacking a killing agent. This contrasts with the findings of Morewood et al. (2002) and de Groot and Nott (2003) on some species of Cerambycidae and Buprestidae. These researchers observed that dry traps with or without insecticide retained fewer insects than traps with soapy water. The larger size, longer legs and greater agility of species of Cerambycidae and Buprestidae may have enabled them to tolerate and escape from dry traps with insecticide (de Groot and Nott 2003) where the much smaller *R. dominica*

could not. Traps with soapy water may be advantageous over dry traps if sex determination of captured *R. dominica* using the squeezing method is desired. We observed that beetles captured in traps with soapy water were softer and less likely to be damaged when squeezing the abdominal body region to extrude beetle genitalia.

We found *R. dominica* populations were higher near grain elevators than in open fields or wooded habitats (Fig. 4). Perhaps this ranking reflects the relative ability of these habitats to sustain *R. dominica* populations. Having wheat, a primary host plant, in the grain bins during the study may either have increased attraction to those locations, or served as a source of beetles. On the other hand, absence of readily available food sources in the open field habitats, and the relatively long distance of these traps from populations of *R. dominica* infested grain, might be responsible for the lower numbers of beetles in open fields. However, the mean numbers of beetles captured in the wooded habitats was higher than the numbers captured in open fields, even though wooded habitats also lacked a stored grain source of *R. dominica*. *R. dominica* rarely, if ever, infests crops in the field prior to harvest, based on thousands of samples collected at harvest in Oklahoma (Edde and Phillips unpublished data). This pattern contrasts to other stored grain pests such as *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) which will commonly infest drying crops in the field prior to harvest (Rees 2004). However, *R. dominica* is known to disperse over distances and has been observed attacking unprotected grain in storage a few weeks after binning (Gates 1995). The source of *R. dominica* attacking newly stored grain remains unknown. We can not rule out the possibility that beetles trapped in the woods in the current study originated from host material other than stored grain. There

are anecdotal reports of *R. dominica* tunneling in various tree species in the wild (Potter 1935; Linsley 1944; Mathew 1987). These beetles have also been reared successfully on several wild fruits and seeds in the laboratory (Wright et al. 1990), indicating that forest habitats may serve as a temporary niches or provide alternative food sources for *R. dominica* when preferred grains like wheat are not available.

Little is known about how *R. dominica* orients to host material not accompanied by pheromones. Preliminary outdoor trapping experiments using whole wheat and wheat extracts have failed to consistently capture *R. dominica*, indicating that the pest may not respond to host plant volatiles from a distance (Edde unpublished data). Fadamiro et al. (1998) obtained similar results with another bostrichid grain pest, *Prostephanus truncatus*. We suspect, as has been proposed for many Scolytidae (Borden 1982), that pioneer male *R. dominica*, dispersing from natal habitats, arrive by chance in grain warehouses where they feed and release aggregation pheromones, to which conspecific males and females are then attracted. The requirement of feeding prior to release of pheromones is well established for *R. dominica* (Mayhew and Phillips 1994, Bashir et al. 2003). As newly arrived males begin to feed, pheromones are produced, thus making grain storage facilities more attractive and easy for dispersing adults to find. It is likely that the higher numbers of beetles captured near grain storages using synthetic pheromones in this study might have resulted from recruitment of beetles already being attracted to these sites by natural pheromone sources in the grain bins. The phenomenon of increased attractiveness of infested food sources is thought to occur in other stored product insects and is well established in several species of bark beetles (Borden 1982; Likhayo and Hodges 2000). Alternatively, the higher number of beetles observed near

grain elevator sites, relative to other habitats tested, might have resulted from emigration of endogenous beetle populations from within the grain bins, i.e. products of earlier infestations and their progeny. However, it is not known if *R. dominica* would leave a source of 'unlimited' food supply, as represented by the grain bins in our study locations, to respond to pheromone signals from our traps outside the bins. Further studies are required to determine which, if any, of these explanations is adequate.

Captures of *R. dominica* in pheromone baited traps were significantly affected by interactions between habitat and trap height. The forest sites had a closed canopy of trees at approximately 6-10 m above the ground. Pheromone-baited traps placed near the vegetation canopies in our wooded sites captured more *R. dominica* than traps placed at lower heights. Information on optimal flight height of dispersing *R. dominica* in different habitats is limited. However, some insect species are known to adopt a predetermined flight height when dispersing, and would avoid obstacles encountered on their flight path (Aborgast 1966). It is probable that the optimal flight height of *R. dominica* responding to pheromone baited traps is below 4 m above the ground; but upon encountering obstacles such as tree trunks in wooded habitats, attempt to fly up and over these. This maneuvering may have brought the beetles into contact with pheromone plumes released from traps placed at higher heights (4 m) and then be captured in them. This hypothesis is supported by the finding that traps placed 1 or 2 m high near grain elevators and open fields performed similarly, and yielded higher trap catches of *R. dominica* than traps placed 4 m high in these habitats. Unlike wooded habitats, trap perimeters in open and grain elevators sites do not have objects that might pose obstacles to approaching beetles.

Traps baited with aggregation pheromones of *R. dominica* captured significantly more females than males, irrespective of the habitat or trap height treatments. This is in agreement with previous observations on several other stored-product and wood boring beetles that utilize male-produced aggregation pheromones (Plarre and Vanderwel 1999; Phillips et al. 2000; Cronin et al. 2000; de Groot and Nott, 2001). One possible explanation is that the primary function of male produced pheromones in *R. dominica* is to attract females as potential mates, but other males exploit the signal for locating assembled females and resources (Phillips 1997; Landolt 1997). In this sense, the aggregation pheromones of *R. dominica* may function more as sex pheromones, as suggested for the stored product pest *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) (White and Chambers 1989), the bark beetle *Dendroctonus terebrans* Zimm. (Phillips et al. 1990), and other Scolytidae (Raffa et al. 1993).

Trap design, trap height and habitat are critical factors that affect responses of *R. dominica* to pheromone-baited traps. Among the trap types tested, the Lindgren trap proved to be most effective in trapping *R. dominica*. Optimum trap height for *R. dominica* varies with habitat. For example, traps should be placed closer to the canopy vegetation in wooded habitats, and from 1 to 2 m high in open habitats. These factors should be considered to optimize monitoring of *R. dominica*.

Acknowledgments

We are grateful to the anonymous reviewers and the editor for their comments and contributions on this manuscript. We thank Phillip Morton, James Robertson, James Metzger and Edmond Bonjour for their technical support. Wendell Burkholder graciously supplied the *Rhyzopertha dominica* pheromones. The USDA CSREES (Risk Avoidance and Mitigation Program) under Agreement No 2000-05385 funded this project.

References cited

Arbogast, R. 1966. Migration of *Agraulis vanillae* in Florida. Flor. Entomol. 49: 141-145.

Arthur, F. 1996. Grain protectants: current status and prospects for the future. J. Stored Prod. Res. 32: 293-302.

Barak, A., W. E. Burkholder, and D. L. Faustini. 1991. Factors affecting the designs of traps for stored-product insects. J. Kans. Entomol. Soc. 63: 466-485.

Bashir, T., R. J. Hodges, L. A. Birkinshaw, D. R. Hall, and D. I. Farman. 2003. Phenotypic plasticity of *Rhyzopertha dominica* pheromone signaling: The effects of different hosts and presence of conspecific females on male produced aggregation pheromone. J. Chem. Ecol. 29: 945-959.

Benhalima, H, M. Q. Chaudhry, K. A. Mills, and N. R. Price. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J. Stored Prod. Res. 40: 241-249.

Borden, J. H. 1982. Aggregation pheromones, pp 74-139. *In* J. B. Mitton, and K. B. Sturgeon (eds.), Bark beetles in North American conifers. University of Texas Press, Austin, Texas.

Borden, J. H., D. W. A. Hunt, D. R. Miller, and K. N. Slessor. 1986. Orientation in forest Coleoptera: an uncertain outcome of response by individual beetles to variable stimuli, pp. 97-109. *In* T. L. Payne, M. C. Birch and C. E. J. Kennedy (eds.), Mechanism in insect olfaction. Clarendon Press, Oxford, UK.

Boucher, T. J, R. A. Ashley, R. G. Adams, and T. F. Morris. 2001. Effect of trap position, habitat, and height on the capture of pepper maggot flies (Diptera: Tephritidae). J. Econ. Entomol. 94: 455-461.

Cogburn, R. R. 1988. Detection, distribution and seasonal abundance of *Sitotroga cerealella* and *Rhyzopertha dominica* as indicated by pheromone-baited adhesive traps, p. 451. *In* Proceedings, XVIII International Congress of Entomology**,** University of British Columbia, 3-9 July 1988**,** Vancouver. B. C. Canada.

Crombie, A. C. 1941. On oviposition, olfactory conditioning and host selection in *Rhyzopertha dominica* Fab. (Insecta, Coleoptera). J. Exp. Bio. 18: 62-79.

Cronin, J. T., J. L. Hayes, and P. Turchin. 2000. Evaluations of traps to monitor southern pine beetle aerial populations and sex ratios. Agric. For. Entomol. 1: 69-76.

De Groot, P., and G. L. DeBarr. 1998. Factors affecting captures of white pine beetle, *Conopthorus coniperda* (Schwarz) (Col., Scolytidae) in pheromones traps. J. Appl. Entomol. 122: 281-286.

De Groot, P., and R. Nott. 2001. Evaluation of six trap designs to capture pine sawyer beetles (Coleoptera: Cerambycidae). Agric. For. Entomol. 3: 107-111.

De Groot, P., and R. Nott. 2003. Response of *Monochamus* (Col., Cerambycidae) and some Buprestidae to flight intercept traps. J. Appl. Entomol. 127: 548-552.

Fadamiro, H. Y., I. Gudrups, and R. I. Hodges. 1998. Upwind flight of *Prostephanus truncatus* is mediated by aggregation pheromone but not food volatiles. J. Stored Prod. Res. 34: 151-158.

Flechtmann, C. A. H., A. L. T. Ottah, and C. W. Berisford. 2000. Comparison of four traps for Ambrosia beetles in Brazilian *Eucalyptus* stands. J. Econ. Entomol. 93: 1701-1707.

Gates, M.W. 1995. Population dynamics of lesser grain borer, rusty grain beetle, and *Cephalonomia waterstoni* in commercial elevators. M.S. thesis, Oklahoma State University Stillwater, Oklahoma.

Hagstrum, D. W., C. Reed, and P. Kenkel. 1999. Management of stored wheat insect pests in the USA. Integrated Pest Man. Rev. 4: 127-142.

Landolt, P. J. 1997. Sex attractant and aggregation pheromones of male phytophagous insect. Am. Entomol. 43: 12-22.

Likhayo, P. W., and R. J. Hodges. 2000. Field monitoring *Sitophilus zeamais* and *Sitophilus oryzae* (Coleoptera: Curculionidae) using refuge and flight traps baited with synthetic pheromone and cracked wheat. J. Stored Prod. Res. 36: 341-353.

Lindgren, B. S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). Can. Entomol. 115: 299-302.

Linsley, E. G. 1944. Natural sources, habitat, and reservoir of insects associated with stored products. Hilgardia 16: 187-224.

Lorini, I., and D. J. Galley. 1999. Deltamethrin resistance in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), a pest of stored grain in Brazil. J. Stored Prod. Res. 35: 37-45.

Mathew, G. 1987. Insect borers of commercially important stored timber in the state of Kerala, India. J. Stored Prod. Res. 23: 185-190.

Mayhew, T. J. and T. W. Phillips. 1994. Pheromone biology of the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). pp 541-544. *In* B. R. Champ, Banks, H. J., and Highley, E. (eds.), Proceedings of the Sixth International Working Conference on Stored-Product Protection. CABI Press. Canberra.

Morewood, W. D., K. E. Hein, K. J. Katinic, and J. H. Borden. 2002. An improved trap for wood-boring insects, with special reference to *Monochamus scutellatus* (Coleoptera: Cerambycidae). Can. J. For. Res. 32: 519-525.

Phillips, T. W. 1997. Semiochemicals of stored product insects: current status and future perspective. J. Stored Prod. Res. 33: 17-30.

Phillips, T. W., P. M. Cogan, and H. Y. Fadamiro. 2000. Pheromones, pp. 273-302. *In* Bh. Subramanyam, and D. W. Hagstrum (eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers. Boston, MA.

Phillips, T.W., J.L. Nation, R.C. Wilkinson, J.L. Foltz, H.D. Pierce and A.C. Oehlschlager. 1990. Response specificity of *Dendroctonus terebrans* (Coleoptera: Scolytidae) to enantiomers of its sex pheromones. Ann. Entomol. Soc. Am. 83: 251-257

Plarre, R., and C. Vanderwel. 1999. Stored-product beetles, pp 149-198. *In* J. Hardie, and A. K. Minks (eds.), Pheromones of non-lepidopteran insects associated with agricultural plants. CABI, UK.

Potter, C. 1935. The biology and distribution of *Rhyzopertha dominica* (Fab.). Tran. Royal Entomol. Soc. 83: 449-482.

Raffa, K.F., T.W. Phillips and S.M. Salom. 1993. Mechanisms and Strategies of Host Colonization by Bark Beetles. pp 103-128. *In* T. Schowalter and G. Filip (eds.),

Interactions Among Bark Beetles, Pathogens and Conifers in North American Forests. Academic Press. London.

Rees, D. 2004. Insects of stored products. CSIRO Publishing. Collingwood, Victoria, Australia.

SAS Institute. 2001. SAS/STAT User's Guide, version 8, SAS Institute Inc., Cary, NC.

Tukey, J.W. 1953. The problem of multiple comparisons. Department of Statistics, Princeton University. Princeton, NJ.

White, P. R., and J. Chambers. 1989. Saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) antennal and behavioral responses to individual components and blends of aggregation pheromone. J. Chem. Ecol. 15: 1015-1031.

Williams, H. J., R. M. Silverstein, W. E. Burkholder, and A. Khorramshahi. 1981. Dominicalure 1 and 2: components of aggregation pheromone from male lesser grain borer *Rhyzopertha dominica* (F). J. Chem. Ecol. 7: 759–780.

Wright, V. F., E. E. Fleming, and D. Post. 1990. Survival of *Rhyzopertha dominica* (Coleoptera, Bostrichidae) on fruits and seeds collected from woodrat nests in Kansas. J. Kans. Entomol. Soc. 63*:* 344-347.

Zar, J. H. 1999. Biostatistical analysis. Prentice-Hall Inc., Englewood Cliffs, NJ.

Table 1. Summary of study sites

Habitat	Geographical Location	Site description
Forest Sites	**I) 36°03'N; 097°10′W 313 m a.s.l.***	**607 ha of naturally regenerating woodland dominated by Eastern Red Cedar *Juniperus virginiana* L. (Cupressaceae), Post oak *Quercus stellata* Wangenh (Fagaceae), and Slippery elm *Ulmus rubra* Muhl. (Ulmaceae) at approximately 348 trees/ha.**
	II) 36°07′N; 097°13′W 300 m a.s.l	**268 ha of naturally regenerating woodland dominated by Chinkapin Oak *Quercus muhlenbergii* Engelm (Fagaceae), Hackberry *Celtis occidentalis* L. (Ulmaceae), Post oak *Quercus stellata* Wangenh (Fagaceae), Loblolly pine *Pinus taeda* L. (Pinaceae) and Redbud *Cercis canadensis* L. (Leguminosae) at approximately 340 trees/ha.**
Open Field	**I) 36°07′N; 097°06W′ 274 m a.s.l.**	**An open field of approximately 34 acres used annually for small-grain breeding, soil fertilizing, variety evaluations and forage research. Several office buildings and grain storage facilities are located on site. More than half of the area was used for variety evaluations of wheat and hay crops during the study period. The remaining area was left fallow.**
	II) 36°07′N; 097°07′W 268 m a.s.l.	**30 acres of open field used annually for hay production. Field was fallow for the duration of the study.**
Grain Elevator	**I) 36°07′N; 097°08′W 276 m a.s.l.**	**A training and grain storage facility having 58 steel bins with combined capacity of 1,143 metric tons of grains but holding approximately 327 metric tons of newly harvested hard red winter wheat *Triticum aestivum* (Herbst) (L.) during the experimental period. In an unrelated study,12 ea. 4.6 metric ton bins at the study site, holding wheat at full capacity, were each infested weekly from May through June 2002 with 500 each of unsexed adults of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Cryptolestes ferrugineus* Stephens (Coleoptera: Laemophloeidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). A mid-sized feed mill is located south and adjacent to the test site.**
	II) 36°09′N; 097°38′W 1035 m a.s.l.	**A grain elevator with four commercial bins with combined capacity of 14,800 metric tons of grains, but held approximately 3,000 metric tons of newly harvested hard red winter wheat *T. aestivum* (L.) during the duration of the study.**

*** a.s.l. above sea level**

Figure legends

Fig. 1. Trap types. (A) Pherocon II trap, (B) Unitrap or bucket trap, (C) Japanese beetle trap and (D) Lindgren multiple funnel trap.

Fig. 2. Mean (±SEM) number of *R. dominica* captured per trap in different trap types baited with aggregation pheromones (DL-1 and DL-2) in Stillwater, OK from 30 May 2000 to 15 June 2000. 4-funnel= Lindgren four-unit funnel traps; Jap. Beetle =Japanese beetle trap; Sticky = Pherocon II sticky trap; Bucket = Unitrap or bucket trap. N = number of replications. Bars with the same letter above them are not significantly different (α= 0.05).

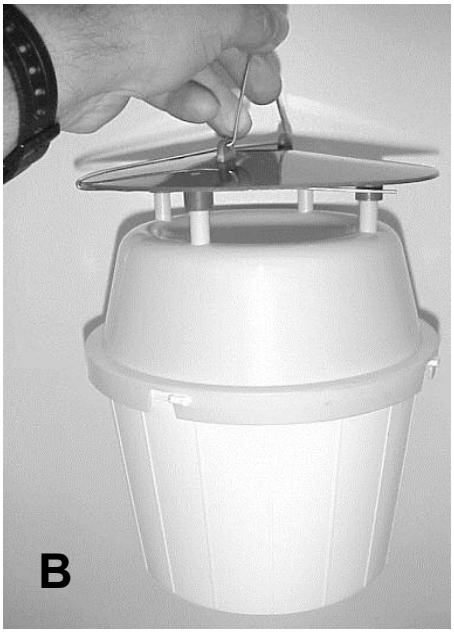
Fig. 3. Mean (±SEM) number of *R. dominica* captured per trap per week in wooded sites, outdoor near grain elevators and open fields at different trap heights in Stillwater, OK from 7 July 2002 to 10 October 2002. Grain = outdoor of grain storage facilities, Wooded = wooded habitat, Open = open field. Bars with the same letter above them are not significantly different (α= 0.05).

Fig. 4. Mean (±SEM) number of *R. dominica* captured per trap per week across locations in wooded sites, outdoor in grain elevators and open field in Stillwater, OK from 7 July 2002 to 10 October 2002. N = number of replications. Bars with the same letter above them are not significantly different (α= 0.05).

Fig. 5. Proportions (±SEM) of *R. dominica* sexes captured per trap in wooded sites, near grain elevators and open fields in Stillwater, OK from 7 July 2002 to 10 October 2002. Grain = outdoor near grain storage facilities, Wooded = wooded habitat, Open = open field. N = number of replications. Bars with the same letter above them are not significantly different (α= 0.05).

Fig. 6. Mean (±SEM) number of *R. dominica* captured in pheromone-baited Lindgren four-unit funnel and Japanese beetle traps with collection cups left blank, dry with insecticide or partially filled with soapy water in Stillwater, OK from 6 September 2004 to 12 September 2004. Funnel = Lindgren four-unit funnel traps; Japanese=Japanese beetle trap; Wet = soapy water; Dry = insecticide and Con = blank collection cups. N = number of replications. Bars with the same letter above them are not significantly different (α= 0.05).

Fig. 1



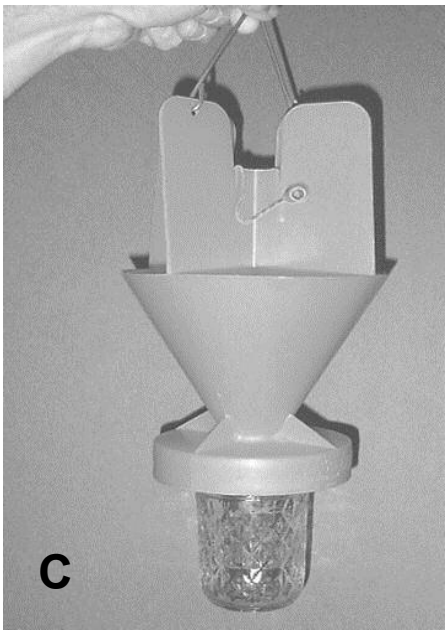


Fig. 2

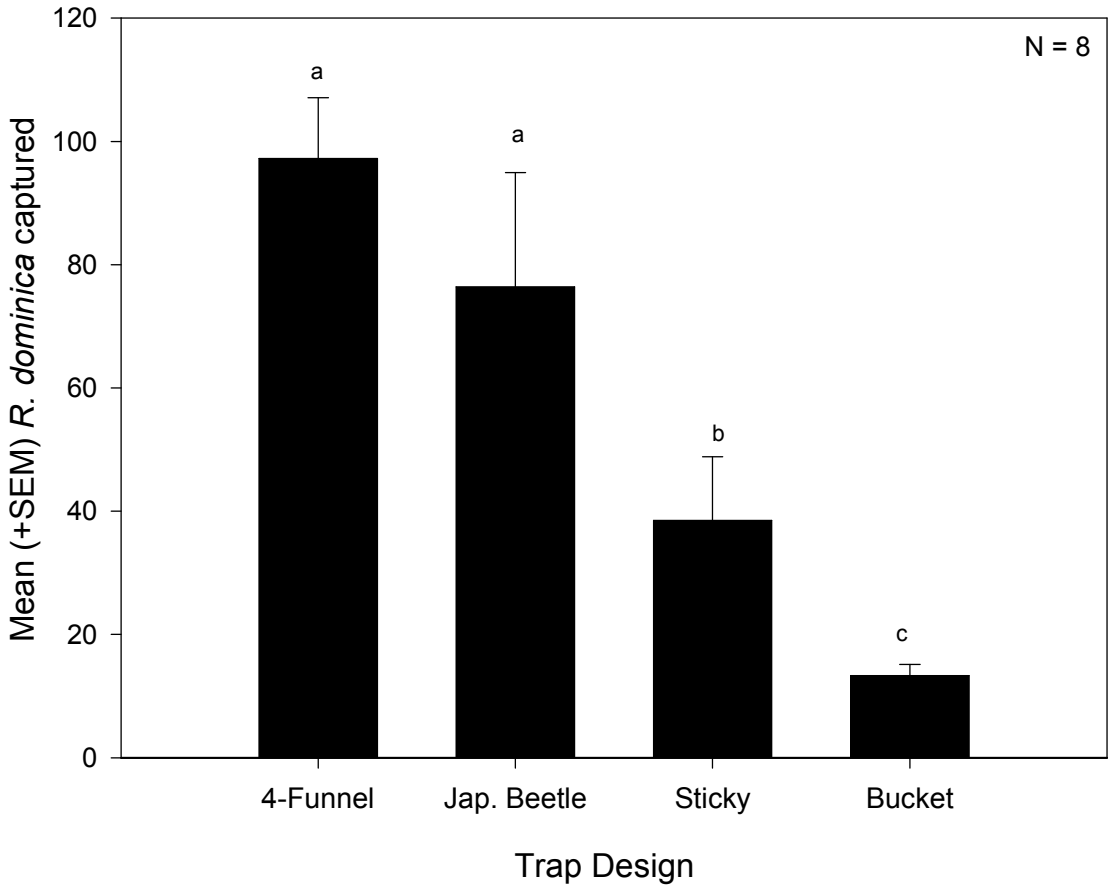

120
100
80
60
40
20
0
Mean (+SEM) R. dominica captured
N = 8
a
a
b
c
4-Funnel
Jap. Beetle
Sticky
Bucket
Trap Design

Fig. 3

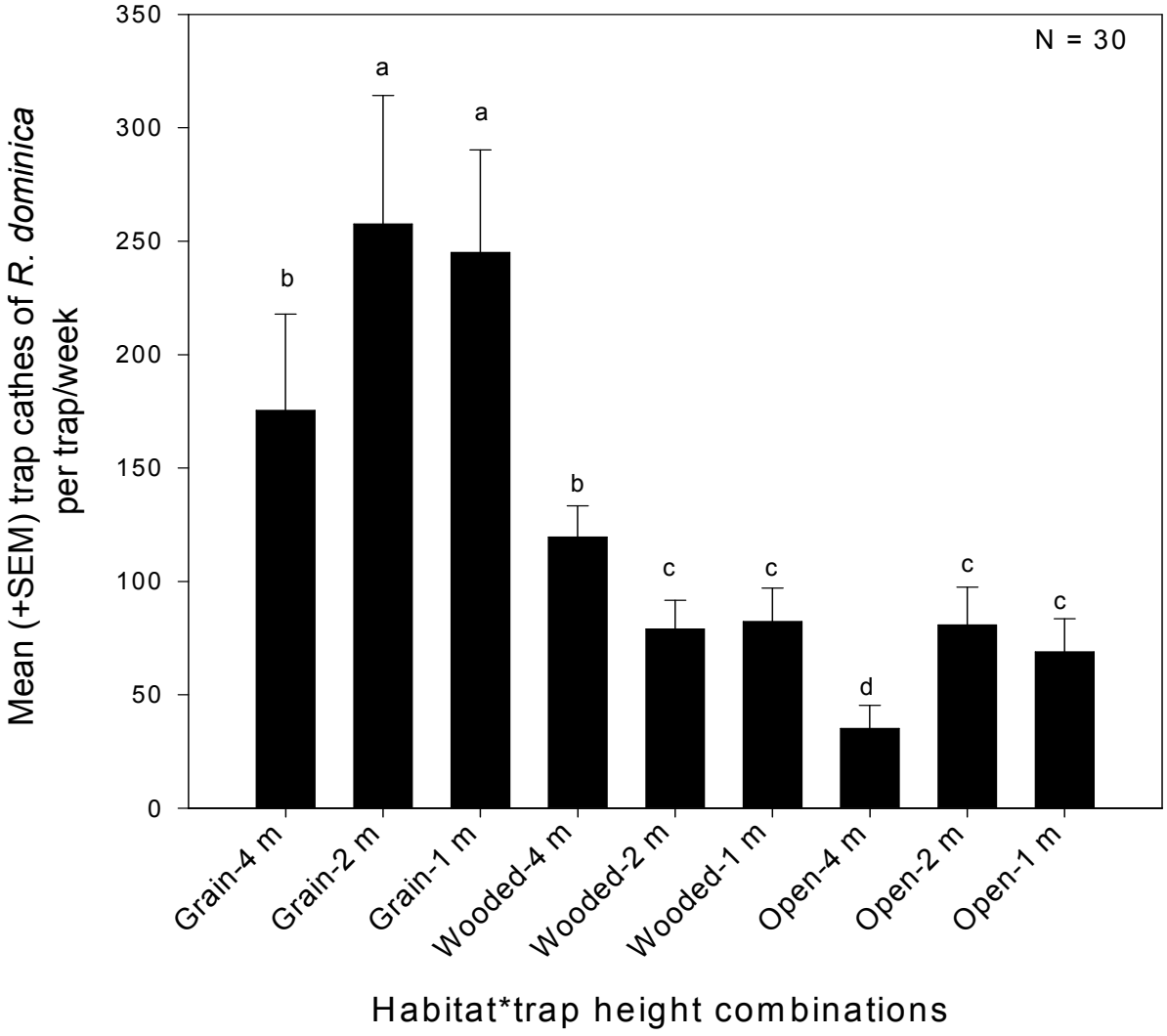

N = 30
Mean (+SEM) trap cathes of R. dominica per trap/week
350
300
250
200
150
100
50
0
a
a
b
b
c
c
d
c
c
Grain-4 m
Grain-2 m
Grain-1 m
Wooded-4 m
Wooded-2 m
Wooded-1 m
Open-4 m
Open-2 m
Open-1 m
Habitat*trap height combinations

Fig. 4

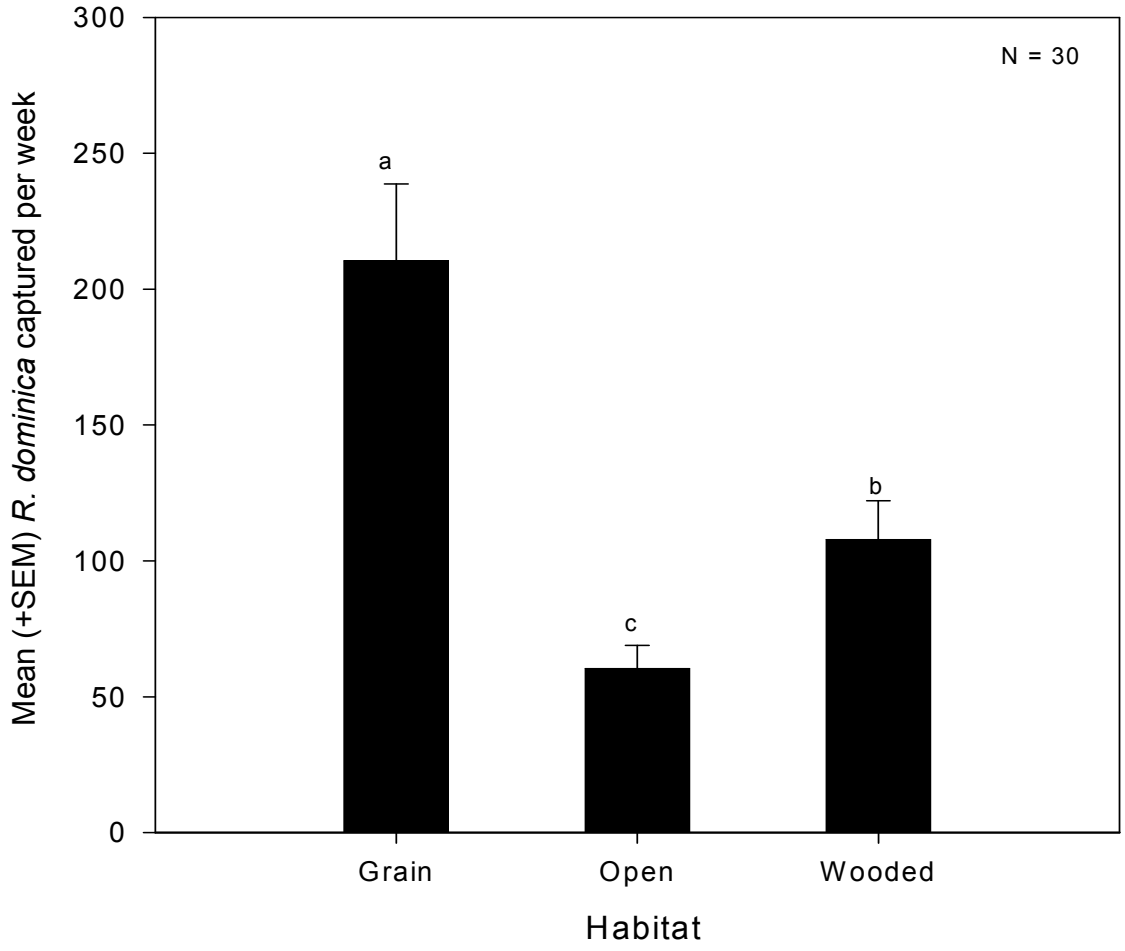

300
250
200
150
100
50
0
N = 30
a
b
c
Mean (+SEM) R. dominica captured per week
Grain
Open
Wooded
Habitat

Fig. 5

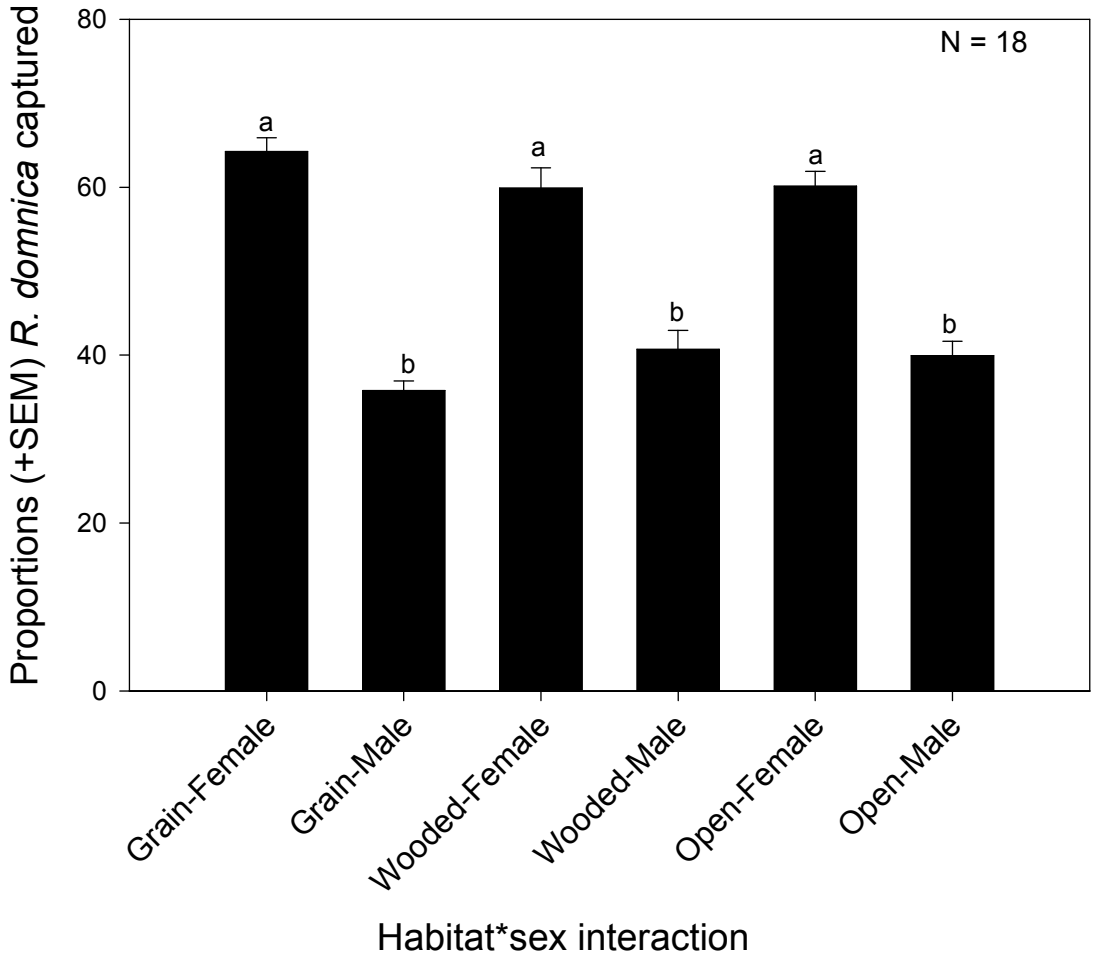
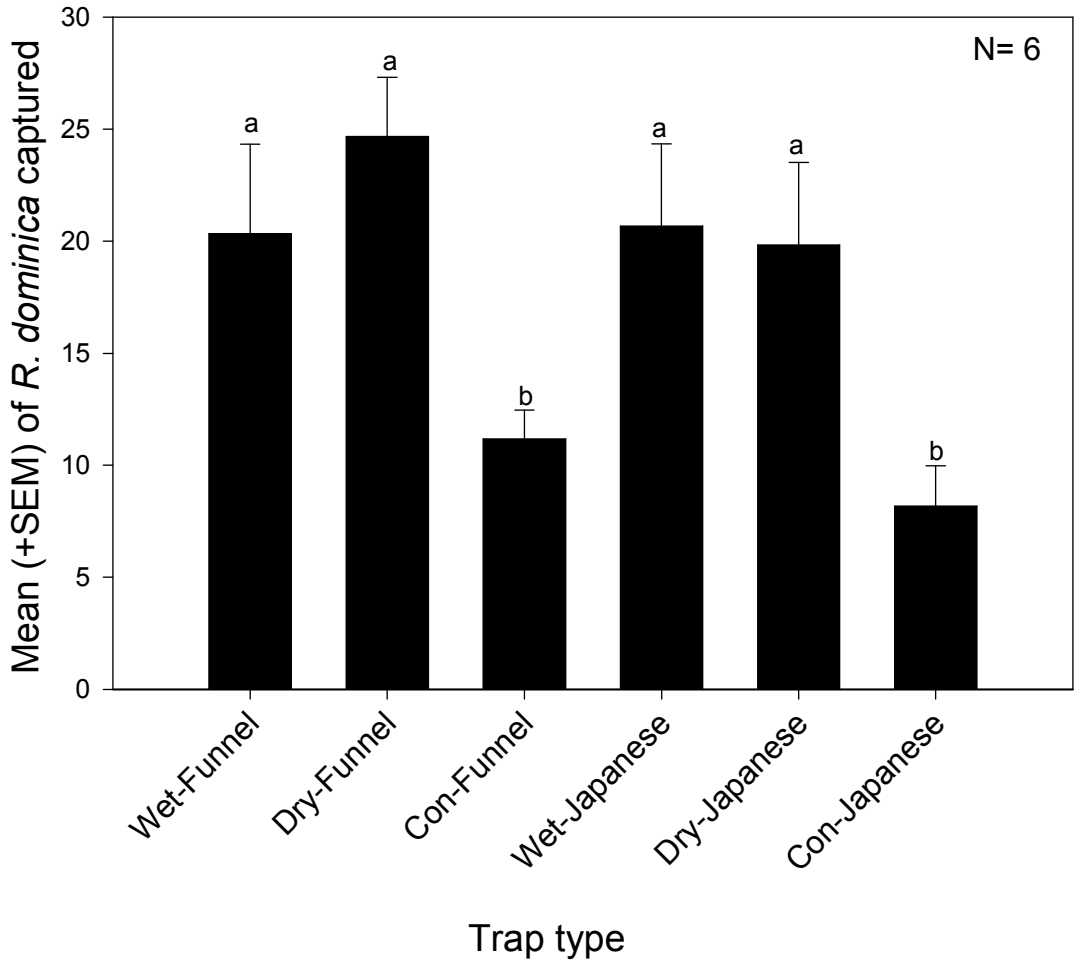

Proportions (+SEM) R. domnica captured
80
60
40
20
0
N = 18
a
b
a
b
a
b
Grain-Female
Grain-Male
Wooded-Female
Wooded-Male
Open-Female
Open-Male
Habitat*sex interaction

Fig. 6


N= 6
Mean (+SEM) of R. dominica captured
30
25
20
15
10
5
0
a
a
b
a
a
b
Wet-Funnel
Dry-Funnel
Con-Funnel
Wet-Japanese
Dry-Japanese
Con-Japanese
Trap type

CHAPTER III

FLIGHT ACTIVITY OF THE LESSER GRAIN BORER, *RHYZOPERTHA DOMINICA* (F.) (COLEOPTERA: BOSTRICHIDAE) IN RELATION TO WEATHER

Environmental Entomology

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Flight Activity of the Lesser Grain Borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), in Relation to Weather

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Abstract

Seasonal flight activity of *Rhyzopertha dominica* near grain elevators and in forest habitats was monitored weekly in central Oklahoma from 2002 to 2005 using Lindgren four-unit multiple funnel traps baited with the synthetic pheromones Dominicalure-1 and Dominicalure-2. Response surface regression was used to model flight activity (*R. dominica* trap data) relative to weather variables (temperature, humidity, amount of rainfall, wind speed) and day length. Overall, the results show more beetle flight activity near grain elevators than in forest sites. Among years, the earliest *R. dominica* flight activity was recorded from 20 to 27 March, and the yearly flight activity ended between 6 to13 November. Seasonal flight activity patterns were similar between habitats; however, in two of the three years of trapping, flight activity generally began at least 1-2 weeks earlier in forest sites as opposed to grain elevators. *R. dominica* were most active during the warmer part of the year. No *R. dominica* were trapped from December through February. About 80 and 86% of the variability in *R. dominica* trap captures was explained by weekly observation of weather variables for grain storage elevators and forest sites, respectively. The weather-based flight activity models for both habitats were validated with independent data.

Keywords: Pheromones, trapping, predictive models, response surface regression

Introduction

THE LESSER GRAIN BORER, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) is a major pest of stored cereals, especially wheat, maize, rice and sorghum in warmer regions of the world (Haines 1991). Adults feed on whole or cracked grain, and larvae develop inside kernels, reducing them to hollow husks. The life history and development rates of *R. dominica* were described in early work (Schwardt 1933, Potter 1935; Crombie 1941). Upon location of suitable food sources, male *R. dominica* release two aggregation pheromones, (S)-(+)-1-methylbutyl (*E*)-2-methyl-2-pentenoate (designated dominicalure-1 or DL-1) and (S)-(+)-1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate (designated dominicalure-2 or DL-2) (Williams et al. 1981). Both sexes respond strongly to a synthetic blend of DL-1 and DL-2 under field and laboratory conditions.

R. dominica is a strong flier (Winterbottom 1922), and extensive trapping studies have shown a characteristic seasonal activity pattern which appears to be linked to weather conditions (Cogburn et al. 1984, Sinclair and Haddrell 1985, Throne and Cline 1994). When infesting stored grain, *R. dominica* is considered an internal feeder, which means that early detection is challenging, and that considerable damage can occur before the beetle infestation is noticed. Unlike some stored-grain pests, *R. dominica* is not known to attack cereals in the field, but has been found infesting grain, in supposedly clean stores, within weeks or months after initial storage (Gates 1995; Hagstrum 2001). This finding suggests possible migration between potentially natural uncultivated habitats and enclosed grain storage facilities. The possibility of migration from wild habitats is supported by the fact that *R. dominica* has been trapped in diverse environments, including woodlands that are substantial distances from grain storage facilities (Cogburn

1988, Edde et al. 2005). Therefore, it may be helpful to consider habitat and climatic factors that cause fluctuation in *R. dominica* densities. An understanding of seasonal activity of *R. dominica* related to weather variables may be used to predict insect problems before they occur in grain elevators, and maximize effectiveness of management decisions such as timing of insecticide application or aerating grain bins.

The first objective of this study was to characterize *R. dominica* seasonal abundance and flight activity pattern near grain storage facilities and in forest sites in central Oklahoma, and the second was to develop models that could be used to predict *R. dominica* flight activity pattern in different habitats in central Oklahoma.

Materials and Methods

Pheromone lures. Pheromone lures used in these experiments were fabricated in our laboratory. Number 11.5 sleeve stoppers (Fisher Scientific, Pittsburgh, PA; referred to here as rubber septa) were first cleaned by soaking overnight in dichloromethane and then allowed to air dry under a fume hood for 24 h. Pheromones were applied to the interior of a rubber septum via a 50% hexane solution containing 5 mg each of DL-1 (chemical purity 95.8%) and DL-2 (chemical purity DL-2 (94%).

***R. dominica* flight activity sampling methods**. Pheromone-baited traps were deployed at four field sites (Table 1; Forest Sites I and II, Grain Elevator Sites I and II) from 7 July through 10 October 2002 and from 15 February 2003 through 30 April 2005. Lindgren four-unit multiple funnel traps (Phero Tech, Delta, British Columbia, Canada) were used in this study. Two traps were maintained at each study site (total eight traps). Traps were hung (approximately 1.7 m above the ground) from vertical polyvinyl chloride pipe

stands that were inserted into the soil, and were spaced 15-20 m apart at each site. Pheromone lures were suspended by a wire at the mid-point inside the second funnel from the bottom of each trap. Traps were serviced and beetle removed at 7-day intervals. New lures were used for each trapping period in each experiment. Captured insects were prevented from escaping by placing pieces of No-Pest® strip (United Industries Corp., St. Louis, MO; active ingredient dichlorvos) in the collection cups. In total, data were collected for 75 weeks yielding 200 weekly observations for grain elevator sites I and II combined and 215 weekly observations for forest sites I and II combined.

Trapping data from February 2003 through April 2005 for site 1 (both forest and grain elevator) were used to characterize seasonal abundance and flight activity pattern of *R. dominica* in the two habitats. However, regression models of the flight activity of *R. dominica* in response to weather variables were constructed using the entire trapping dataset (July 2002 through October 2002, and February 2003 through April 2005), but were limited to trap catch data from the week when beetles were first caught to the cessation of beetle flight activity for each trapping year.

Meteorological data. Weather data were obtained on minimum and maximum air temperature (°C), minimum and maximum relative air humidity (%), minimum and maximum wind speed (km/hr), and rainfall (mm) from the Oklahoma meteorological stations near each study site (http://www.mesonet.org/premium) (Table 1). Although, weather stations were located near forest sites they were situated outside the mature forests in small clearings. Measurements of day length were obtained from the Astronomical Applications Department of the U.S. Naval Observatory (http://aa.usno.navy.mil).

Analyses. Because trapping was performed on a weekly basis, weekly means of the selected weather variables and daylengths were used in the analysis. Data analyses were performed using SAS Version 8 (SAS Institute 2001). A normal probability plot was used to determine normality and homogeneity of variance of weekly *R. dominica* trap capture data. Based on the results, the data were log-transformed, using the Log (X +1) transformation (Zar 1999).

A PROC MIXED for repeated measure model was used with an autoregressive correlation variance structure to analyze differences between forest and grain elevator site in beetle catches per trap across time (weekly data from February 2003 through April 2005). Weather data variables within habitat and between the two habitats for 2003 and 2004 were evaluated with independent t-tests (PROC TTEST).

Multiple backward stepwise regression (Neter et al. 1996) was performed to model *R. dominica* trap catch to determine if data from forest and grain elevator sites could be combined to present a single flight model for the species in both habitats. Habitat type was included in the model with the use of an indicator variable and interaction terms with all the explanatory variables, yielding a total of 17 parameters in the model. The assumption was that if forest and grain elevator habitats gave the same basic regressions, then most, if not all the interaction terms in the parameters would be removed from the regression model. To assess the potential quadratic nature of each explanatory variable, a response surface regression (PROC RSREG) was used to analyze the relationship between weather variables and weekly flight activity of *R. dominica*. In order to reduce the numbers of parameters without sacrificing model accuracy, the parameters generated in the response surface regression were subjected to single step

evaluation to eliminate model parameters with no contribution to the model fit. The parameters were individually evaluated in a PROC REG analysis to calculate the contribution of each parameter by using the coefficient of determination (R^2) as a criterion associated with model fit. A predictive model for *R. dominica* flight activity was chosen when further addition of a parameter resulted in no improvement (Freund and Littell 1991, Nansen 2001, 2004).

Validation. Validation of the predictive capability of the regression models was accomplished with trapping observations not used in fitting the original models. Trapping for validation purposes was conducted from 2 April through 2 July 2005 at grain elevator sites I-IV and forest sites I and II (Table 1). Two or three traps were deployed weekly at each of the grain elevator site, and 4-6 traps deployed weekly at each of the forest sites. In total, there were 134 and 129 weekly observations for validation purposes for the flight activity model for grain elevator and forest sites, respectively. Weather data for the validation data were obtained from meteorological stations near the study sites (Table 1). Pheromone lures, Lindgren funnel traps and trap spacing were identical to those used to construct the models.

Results

Analysis of weather data indicated that conditions were similar at grain elevator site I and forest site I in 2003, with exception of minimum wind speed, which was significantly higher in forested area than in grain elevator site (t-test = 6.07; df = 129; $P < 0.001$) (Table 2). However, in 2004, significantly larger means were observed for forest habitat for maximum humidity (t-test = 3.21; df = 99.2; $P < 0.01$), minimum humidity (t-test =

1.95; df = 146; $P = 0.05$), maximum wind speed (t-test = 2.96; df = 146; $P < 0.01$) and minimum wind speed (t-test = 6.98; df = 137; $P < 0.001$). Whereas, weekly mean of maximum temperature was higher around grain elevator relative to forest habitat for same year (t-test = -2.73; df = 146; $P < 0.01$). Minimum temperature, day length, precipitation and proportion of days with rain were not significantly different among habitats in 2004. There was no evidence of statistical differences in weather conditions at the grain elevator site between 2003 and 2004 trapping studies, except in maximum relative humidity, which was significantly higher (t-value = 3.15; df = 138; $P < 0.01$) in 2003 than 2004. For the forest habitat, three weather variables were markedly higher in 2003 than in 2004 trapping seasons: minimum humidity (t-test = -2.13; df = 138; $P < 0.05$), minimum wind speed (t-test = -2.43; df = 138; $P < 0.05$) and rainy days (t-test = -2.06; df = 138; $P < 0.05$),

Flight activity of *R. dominica* near grain elevator and forest site. In total, 36,822 *R. dominica* (all traps at grain elevator site I and forest site I combined) were caught in February 2003 through April 2005. Of the total trap catches, 23,934 and 12,888 individuals were caught at grain elevator site and forest site, respectively. Repeated measure ANOVA confirmed that *R. dominica* trap catches near grain storage elevator were significantly higher than in forest site ($F = 12.41$; df = 1, 46; $P < 0.01$). The analysis also showed that *R. dominica* flight activity was significantly different among weeks within trapping sites ($F = 10.13$; df = 35, 180; $P < 0.001$). Of the total trap catches, 19,148 and 17,674 individuals were caught in 2003 and 2004, respectively, but there was no significant difference in trap captures between years ($F = 0.71$; df = 1, 40; $P = 0.41$). In general, climatic conditions did not cause noticeable differences in yearly *R. dominica*

population activity across habitats, but within year and habitat, variation in beetle flight activity was largely governed by short time (weekly) differences in weather conditions.

Analysis of *R. dominica* trap catches within habitats indicated that the number of *R. dominica* captured in 2003 and 2004 were not significantly different for grain elevator site (t-test = 1.62; df = 205; $P = 0.11$), or forest site (t-test = -1.39; df = 205; $P = 0.17$).

Seasonal patterns of *R. dominica* flight activity at site I (both grain elevator site and forest site) are presented in Fig. 1. In 2003, the first *R. dominica* was captured during the week of 4 - 11 April in forest site, but not until a week later at grain elevator site. A similar pattern was observed in 2004, with the first flight activity recorded in the week of 20 - 27 March in forest site, but no *R. dominica* were captured until two weeks later (3 - 10 April) at grain elevator site. However, commencement of *R. dominica* flight activity in forest and at the grain elevator site in 2005 occurred during the same week, 27 March - 2 April in both trapping sites. The cessation of *R. dominica* flight activity in 2003 was recorded on the week of 24 - 31 October and 31 October - 7 November at grain elevator and forest site, respectively. However, cessation of beetle activity in forest and near grain elevator sites in 2004 was observed during the same week of 6 - 13 November at both study sites. In general, the flight activity seemed to be tri-modal for each year of this study such that peaks of trap captures occurred in May, Sept and early October.

Influence of meteorological conditions on activity. Results of the stepwise regression analysis to determine if data from forest and grain elevator sites could be combined to present a single flight model for the species in both habitats showed that 67% of the terms that included the common variable failed to be removed from the model, indicating significant differences on the effect of weather conditions on *R. dominica*

flight activity between the two habitats. Therefore, we decided to keep the forest and grain elevator regressions separate.

Forty-four parameters, including 8 linear responses, 8 quadratic responses and 28 linear interactions, were generated with eight weather variables in the full response surface regression model of *R. dominica* flight activity at grain and forest sites. Results of the single step exclusion of model parameters with no contribution to the model are given in Fig. 2. We found that 80 and 86% of the total variance was explained when the first 33 parameters were included in the model for *R. dominica* flight activity at grain elevator or forest sites, respectively. Including the remaining 11 parameters resulted in no improvement to the model fit for *R. dominica* flight activity in either habitat. We decided, therefore, to base the models on the first 33 parameters because they explained most of the variance with the fewest parameters. Apparently, *R. dominica* flight activity near grain elevators was largely dependent on linear responses to maximum air temperature (Fig. 2), accounting for 71% of the total variance explained. Primary contributors to model fit in forest habitats were linear interactions between wind speeds and temperatures, of which linear interactions between maximum wind speed and minimum air temperatures was the most important and accounted for about 31% of the total variation of flight activity (Fig. 2). Model parameters and associated coefficients for *R. dominica* flight activity at grain elevator and forest sites are given in Table 3 and Table 4, respectively.

Model validation. Linear regression analysis of the observed vs. predicted *R. dominica* trap catches at grain elevator and forest validation sites, respectively, are summarized in Fig. 3. Results confirmed that the regression model developed for grain elevator site

could be used to explain flight activity at elevator site I ($F = 138.2$, df = 38, $P < 0.001$), elevator site II ($F = 88.3$, df = 49, $P < 0.001$), elevator site III ($F = 35.0$; df = 15; $P < 0.001$) and IV ($F = 38.9$, df = 40, $P < 0.001$). Similarly, the model for forest habitats was valid for forest site I ($F = 5.8$, df = 44, $P < 0.05$) and forest site II ($F = 49.6$, df = 83, $P < 0.001$), although these lacked the higher level of prediction found for the elevator model.

Discussion

The similarities in *R. dominica* flight activity between grain elevator and forest habitats suggests that temporal variation in beetle activity between or within habitats may be more related to weather conditions expressed over a short-term, or weekly, basis, rather than on longer term seasonal or yearly scales. Therefore, it is possible to predict differences in *R. dominica* flight activity in the two habitats based on just one year of data. The lack of year-to-year variation in *R. dominica* flight activity within habitats observed in the present study differs from the yearly variation in flight activity found in other stored-product insect pests, such as *Cryptolestes ferrugineus* (Nansen et al. 2004,) and *P. truncatus* (Borgemeister et al. 1997). The authors of these two latter studies collected more than two years of flight activity, data and attributed yearly variation to macro-climatological factors. Our conclusion, of a lack of yearly variation in *R. dominica* flight activity, is based on just two years of trapping data and should be considered preliminary until data from several years can be collected

Previous reports on seasonal flight activity of *R. dominica* included those by Schwitzgebegel and Walkden (1944), Cogburn et al. (1984), Sinclair and Haddrell (1985) and Throne and Cline (1994). Similar to our findings, these early reports showed that

relatively high *R. dominica* flight activity occurred during the warmer times of the year (July through September). However, our findings on commencement and cessation of *R. dominica* flight activity differ from observations by Schwitzgebegel and Walkden (1944) and Throne and Cline (1994). For example, during a survey to monitor migration of stored grain insect pests into wheat bins in Kansas, Schwitzgebegel and Walkden (1944) caught the first *R. dominica* between 13 - 15 May in sticky traps placed at ventilator openings of grain bins. Throne and Cline (1994) in a two year study to examine activity of various stored-product insect pests outside grain elevators observed that *R. dominica* was active year-round in South Carolina. Our traps were baited with *R. dominica* pheromones and, thus were probably more attractive than the passive sticky traps used in these previous studies, which could explain our ability to detect *R. dominica* flight earlier in the spring than did Schwitzgebegel and Walkden (1944) in Kansas. However, we did not capture any *R. dominica* in either of the trapping sites from December through February during the course of our study, which is a time of the year when daily high temperatures rarely, if ever, exceeded 10.9 ± 6.9°C. Thus, we propose the threshold temperature for flight of *R. dominica* in central Oklahoma to be on average 10.9°C. The South Carolina sites used by Throne and Cline (1994) were southerly in distribution and climate, and temperatures were presumably warmer there in the winter time than in Oklahoma; hence, insects like *R. dominica* are likely to be active year round in South Carolina.

Similar flight activity patterns of *R. dominica* observed between forest and grain storage suggest possible dependency between the two habitats in sustaining *R. dominica* populations. It is likely that the relatively early flight activity recorded in forest compared

to grain storage site (Fig. 1), the time lag between flight occurrence in wooded habitat and grain storage, as well as the first and second peaks of flight activity in forest and grain elevator sites represents migration of the insect from over-wintering sites in the forest in search of preferred food sources at grain elevators. Likewise, the third, but brief burst of flight activity observed in both habitats might represent migration of the insect from grain storage facilities back to the forest for over-wintering. Synchrony of *R. dominica* flight activity patterns between forest and grain storage underscores the need for further studies on the dispersal behavior and the effects of habitat quality and interactions between *R. dominica* metapopulations. *R. dominica* is a strong flier, thus, an ability by adult beetles to migrate between uncultivated natural habitats and grain storages may confer the ability to colonize new or previously infested grain storage, and which may have important consequence for local and regional population dynamics and inter-population gene flow (Briers et al. 2003),

Results of the stepwise regression analysis to determine if data from forest and grain elevator sites could be combined to present a single flight model for the species in both habitats confirmed that the influence of weather conditions on *R. dominica* flight activity varies between the two habitats. Habitat-based models of *R. dominica* flight activity may provide better understanding of the underlying interactions between inherent habitat quality and climatic perturbations, and enable accurate characterization of the relationships between trap catches, flight behavior, and population density. The observed interhabitat variation in the relationship between *R. dominica* flight activity and weather variables presumably reflects differences in exposure of the habitats. The grain elevator sites used in our study, which are typical of grain elevator sites in central Oklahoma, are

opened, nonforested areas that could have extremes in climatic conditions on a more frequent basis than the forest sites due to lack of tree cover. For example, Saptomo et al. (2004) observed that a greater amount of energy may be dissipated into heat in open conditions, which may lead to relatively higher environmental temperature and wind speed in open conditions than in fields covered by vegetation.

The flight activity models developed in this study confirm that weather variables explained most of the variation in *R. dominica* trap captures for grain elevator and forest sites. The remaining unexplained variations must be attributed to factors not included in the models, and to stochasticity. For example, because our trap catch data were collected on a weekly basis, we also summarized data on weather variables as weekly means. These computations may have resulted in loss of precision in the regressions that resulted from unexplained variance due to summarizing meteorological data as weekly means (Briers et al. 2003). The model for *R. dominica* flight activity for grain elevator sites gave a good prediction of beetle flight at the four different validation grain elevator sites during 2005, with regression values ranging from 0.51 to 0.84 (Fig. 3). The validations for forest sites were also good, but with lower regression values of 0.12 and 0.37. These validations (R^2 values) are lower than those from the model fitting procedures which is consistent with linear regression theory (Neumann et al. 2003). Models describing the limits of flight activity under different climatic conditions will aid in the management of stored grain by predicting the time when migrant insect pests are a threat. Our flight activity models accurately predicts when no flight activity of *R. dominica* would occur in both types of habitats, as well as when large flights (>500 near grain elevators; >220 in forested areas) would occur over short time scales (i.e. weekly interval). These models

could thus be useful to assist farmers and grain elevator operators in predicting the onset of insect problems and for proper timing of management practices such as fumigant insecticide application or aerating grain bins, which are most effective after pest immigration has ceased.

Acknowledgements

We thank Richard Berberet and Kristopher Giles for reviewing an early draft of the manuscript. We are also grateful to Phillip Taylor who helped with identification of trees at the experimental sites. James Barrett Robertson assisted with counting of insects. Wendell Burkholder, USDA, Madison, WI (retired) graciously supplied *R. dominica*. The USDA CSREES Risk Avoidance and Mitigation Program under Agreement No 2000-05385 and the Oklahoma State University Integrated Pest Management Mini-Grant Program funded this project.

References cited

Briers, R. A., H. M. Cariss, and J. H. Gee. 2003. Flight activity of adult stoneflies in relation to weather. Ecol. Entomol. 28: 31-40.

Borgemeister, C., W. C. Meikle, D. Scholz, C. Adda, P. Gegbey, and R. H. Markham. 1997. Seasonal and climate variables influencing the annual flight cycle of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and its predator *Teretriosoma nigrescens* (Coleoptera: Histeridae) in southwestern Benin. Bull. Entomol. Res. 87: 239-246.

Cogburn, R. R. 1988. Detection, distribution and seasonal abundance of *Sitotroga cerealella* and *Rhyzopertha dominica* as indicated by pheromone-baited adhesive traps, p 451. *In* Proceedings, XVIII International Congress of Entomology**,** University of British Columbia, 3-9 July 1988**,** Vancouver. B. C. Canada.

Cogburn, R. R., W. E. Burkholder, and H. J. Williams. 1984. Field test with the aggregation pheromone of the lesser grain borer (Coleoptera: Bostrichidae). Environ. Entomol. 13: 162-166.

Crombie, A. C. 1941. On oviposition, olfactory conditioning and host selection in *Rhyzopertha dominica* Fab. (Insecta, Coleoptera). J. Exp. Bio. 18: 62-79.

Edde, P. A., T. W. Phillips, and M. D. Toews. 2005. Responses of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromones as influenced by trap design, trap height and habitat. Environ. Entomol. (in press).

Freund, R. J., and R. C. Littell. 1991. SAS system for regression. SAS Institute, Cary, NC.

Gates, M.W. 1995. Population dynamics of lesser grain borer, rusty grain beetle, and *Cephalonomia waterstoni* in commercial elevators. M.S. thesis, Oklahoma State University Stillwater, Oklahoma

Hagstrum, D. W. 2001. Immigration of insects into bins storing newly harvested wheat on 12 Kansas farms. J. Stored Prod. Res. 37: 221-229.

Haines, C. P. (ed.) 1991. Insect and Arachnid of Tropical Stored Products: Their Biology and Identification. Natural Resources Institute, Chartham, UK.

Nansen, C., S. Korie, W. G. Meikle, and N. Holst. 2001. Sensitivity of *Prostephanus truncatus* (Coleoptera: Bostrichidae) flight activity to environmental variables in Benin, West Africa. Environ. Entomol. 30: 1143-1143.

Nansen, C., E. L. Bonjour, M. W. Gates, T. W. Phillips, G. W. Cuperus, and M. E. Payton. 2004. Model of *Cryptolestes ferrugineus* flight activity outside commercial steel grain bins in Central Oklahoma. Environ. Entomol. 33: 426-434.

Neumann, D. W., B. Rajagopalan, and E. A. Zagona. 2003. Regression model for daily maximum stream temperature. J. Environ. Eng. 129: 667-674.

Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. Applied linear regression models. Richard D. Irwin Inc. Homewood, IL.

Potter, C. 1935. The biology and distribution of *Rhizopertha dominica* (Fab.). Trans. Royal Entomol. Soc. 83: 449-482.

Saptomo, S. K., Y. Nakano, K. Yuge, and T. Haraguchi, 2004. Observation and simulation of thermal environment in a paddy field. Paddy and Water Environ. 2: 73-82.

SAS Institute. 2001. SAS/STAT User's Guide, version 8, SAS Institute Inc., Cary, NC.

Schwardt, H. H. 1933. Life history of the lesser grain borer. J. Kans. Entomol. Soc. 2: 61-66.

Schwitzgebegel and Walkden. 1944. Summer infestation of farm-stored grain by migrating insects. J. Econ. Entomol. 37: 21-24.

Sinclair, E. R., and R. L. Haddrell. 1985. Flight of stored products beetles over a grain farming area in southern Queensland. J. Aust. Entomol. Soc. 24: 9-15

Throne, J. E., and L. D. Cline. 1994. Seasonal flight activity and seasonal abundance of selected stored-product Coleoptera around grain storages in South Carolina. J. Agric. Entomol. 11: 321-338.

Williams, H. J., R. M. Silverstein, W. E. Burkholder, and A. Khorramshahi. 1981. Dominicalure 1 and 2: components of aggregation pheromone from male lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). J. Chem. Ecol. 7: 759-781.

Winterbottom, D. C. 1922. Weevil in wheat and storage of grain in bags. A record of Australian experience during the war period (1915 to 1919). South Australian Government, Australia.

Zar, J. H. 1999. Biostatistical analysis. Prentice-Hall Inc., Englewood Cliffs, NJ.

Table 1. Summary of study sites and nearest weather stations in central Oklahoma

Habitat and location	Weather station	Site description
Forest site I 36°03'N; 097°10′W	36°03'N; 097°12′W	607 ha of woodland dominated by *Juniperus virginiana* L. (Cupressaceae), *Quercus stellata* Wangenh (Fagaceae), *Ulmus rubra* Muhl. (Ulmaceae) at approximately 348 trees/ha. Pheromone-baited traps were installed at least 25 m from the forest edge
Forest site II 36°45′N; 097°13′W	36°45′N 097°15′W	268 ha of woodland dominated by *Quercus muhlenbergii* Engelm (Fagaceae), *Celtis occidentalis* L. (Ulmaceae), *Quercus stellata* Wangenh (Fagaceae), *Pinus taeda* L. (Pinaceae) and *Cercis Canadensis* L. (Fabaceae) at approximately 340 trees/ha. Pheromone-baited traps were installed at least 15 m from the forest edge.
Grain elevator site I 36°07′N; 097°08′W	36°07′N 097°05′W	A small grain storage facility having 58 steel bins with combined capacity of 1,143 metric tons of grains but holding approximately 327 metric tons hard red winter wheat *Triticum aestivum* (L.) for most of the study period. A mid-sized feed mill is located south and adjacent to the test site. Pheromone-baited traps were installed at least 15 m away from the grain bins at this site, as well as in grain elevator sites II-IV below.
Grain elevator site II 36°26′N; 097°67′W	36°07′N 097°36′W	Commercial grain elevator with combined capacity of 13,432 metric tons of grains, but held approximately 8,108 metric tons of hard red winter wheat for most part of the study period.
Grain elevator site III 36°14'N; 095°44'W	36°8'N 095°27'W	Commercial grain elevator with capacity for 108,000 metric tons of grains, but held about 86,000 and 15,000 metric tons of hard red winter wheat and soybean (*Glycine max* L.), respectively, during the study period. About 6,000 metric tons of milo *Sorghum vulgare* Pers. and barley *Hordeum jubatum* L. were also stored at the site.
Grain elevator site IV 36°09′N; 097°38′W	36°07′N 097°36′W	Commercial grain elevator with combined capacity of 14,800 metric tons of grains, but held approximately 3,000 metric tons of hard red winter wheat for most part of the study period.

Table 2. Mean ±SE of weekly weather variables during trapping periods near grain elevator site I and forest site 1 in central Oklahoma from January 2003 through December 2004[1]

Habitat	Maximum humidity (%)	Minimum humidity (%)	Maximum temp. (°C)	Minimum temp. (°C)	Maximum wind speed (km/h)	Minimum wind speed (km/h)	Day length (decimal hr)	Rainfall (mm)
				2003				
Gain elevator	91.8±0.6 A	44.8±1.3	27.6±0.8	14.4±0.8	25.1±0.5	1.9±0.2 b	13.1±0.2	1.5±0.3
Forest	90.9±0.7	45.5±1.4 B	27.5±0.8	14.5±0.7	26.5±0.5	3.6±0.2 aA	13.1±0.2	1.7±0.3
				2004				
Grain elevator	87.7±1.0 bB	46.3±1.0 b	28.1±0.6 a	15.0±0.6	24.5±b	2.2±0.2 b	12.9±0.2	2.0±0.3
Forest	91.8±0.5 a	49.5±1.2 aA	25.8±0.7 b	14.1±0.6	26.7±0.6 a	4.4±0.3 aB	12.9±0.2	2.1±0.3

[1] Means with different lower-case letters represent significant differences between habitats within year, and those with different upper-case letters represents comparison within habitat between year. (PROC TTEST; $P = 0.05$)

Table 3. Parameters included in model of weather variables for *R. dominica* flight activity near grain elevators.

Variable	df	Parameter estimate	t-value	*P*	Model R^2
Intercept	1	207.4124	3.61	0.0004	0.80
Linear					
HX	1	-3.0750	-5.52	<.0001	
RA	1	6.4917	4.28	<.0001	
HM	1	-1.9583	-3.92	0.0001	
TX	1	-9.9306	-3.83	0.0002	
DL	1	8.3412	2.99	0.0031	
TM	1	8.2780	3.00	0.0031	
WM	1	-4.0987	-1.99	0.0483	
Quadratic					
DL*DL	1	-0.3653	-2.99	0.0032	
TX*TX	1	0.0391	2.41	0.0168	
RA*RA	1	-0.0091	-1.16	0.2481	
TM*TM	1	0.0152	0.72	0.4699	
Interaction					
TX*HX	1	0.1310	5.04	<.0001	
HX*HM	1	0.0237	4.67	<.0001	
TX*WX	1	-0.0661	-4.51	<.0001	
TM*HX	1	-0.1170	-4.50	<.0001	
WX*DL	1	0.0945	4.32	<.0001	
TM*WX	1	0.0538	4.26	<.0001	
RA*HM	1	-0.0368	-4.06	<.0001	
RA*WX	1	-0.0364	-3.48	0.0006	
TM*RA	1	0.1239	3.10	0.0022	
TX*RA	1	-0.1190	-3.12	0.0021	
WM*WX	1	-0.0460	-3.1	0.0022	
WM*HX	1	0.0593	3.07	0.0024	
WM*TX	1	0.1997	3.00	0.0030	
TM*DL	1	0.1709	2.92	0.0039	
WM*DL	1	-0.2200	-2.89	0.0043	
WM*TM	1	-0.1899	-2.84	0.0050	
TX*DL	1	-0.1228	-2.24	0.0264	
TX*TM	1	-0.0555	-1.6	0.1104	
HX*RA	1	-0.0242	-1.45	0.1486	
WX*HM	1	-0.0027	-0.87	0.3867	
WM*HM	1	-0.0054	-0.37	0.7118	
RA*DL	1	0.0036	0.12	0.9043	

DL= day length (decimal hour), HM= min. relative humidity (%), HX= max. relative humidity (%), RA= amount of rainfall, TM= min. air temperature (^{0}C), TX= max. air temperature (^{0}C), WM= min wind speed (km/h), WX= max. wind speed (km/h).

Table 4. Parameters included in model of weather variables for *R. dominica* flight activity in forest habitats.

Variable	df	Parameter estimate	t-value	*P*	Model R^2
Intercept		-38.0337	-1.2	0.2333	0.86
Linear					
TM	1	3.4787	4.14	<.0001	
RA	1	-1.1360	-3.88	0.0001	
HX	1	1.7561	2.82	0.0054	
DL	1	-7.3317	-2.92	0.0039	
WX	1	0.9396	2.51	0.0130	
TX	1	-1.4493	-2.01	0.0459	
HM	1	-0.3840	-1.26	0.2082	
Quadratic					
TM*TM	1	0.1089	5.23	<.0001	
TX*TX	1	0.0530	2.66	0.0084	
DL*DL	1	0.2248	2.66	0.0085	
HX*HX	1	-0.0093	-2.08	0.0394	
WX*WX	1	0.0045	1.34	0.1814	
HM*HM	1	-0.0008	-0.6	0.5496	
Interaction					
WM*TM	1	-0.2251	-6.49	<.0001	
WM*TX	1	0.1707	5.05	<.0001	
RA*DL	1	0.0836	4.17	<.0001	
WM*HX	1	-0.0385	-4.2	<.0001	
TM*WX	1	0.0726	4.3	<.0001	
TX*RA	1	0.0448	3.98	0.0001	
WM*WX	1	-0.0381	-3.62	0.0004	
TM*TX	1	-0.1765	-4.53	<.0001	
WM*DL	1	0.1580	3.39	0.0009	
TM*RA	1	-0.0540	-3.5	0.0006	
TX*WX	1	-0.0509	-3.31	0.0011	
TM*DL	1	-0.2337	-3.21	0.0016	
TX*DL	1	0.1993	3.08	0.0024	
WM*HM	1	0.0263	3.04	0.0028	
WX*DL	1	-0.0563	-2.74	0.0068	
WX*RA	1	-0.0125	-2.62	0.0096	
TX*HX	1	-0.0051	-1.54	0.1247	
HM*DL	1	0.0111	0.87	0.3881	
HM*HX	1	0.0032	0.72	0.4738	
WX*HM	1	-0.0012	-0.38	0.7049	

DL= day length (decimal hour), HM= min. relative humidity (%), HX= max. relative humidity (%), RA= amount of rainfall, TM= min. air temperature (^{0}C), TX= max. air temperature (^{0}C), WM= min wind speed (km/h), WX= max. wind speed (km/h).

Figure legends

Fig. 1. Weekly trap catches of *R. dominica* near forest site I and grain elevator site I from February 2003 through April 2005 in central Oklahoma. Data are means of two traps per site.

Fig. 2. Stepwise exclusion of the least model parameters (lowest R^2 value) in the regression analysis of *R. dominica* flight activity near grain elevators and forest sites in central Oklahoma. DL= day length (decimal hour), HM= minimum relative humidity (%), HX= maximum relative humidity (%), RA= amount of rainfall, TM= minimum air temperature (°C), TX= maximum air temperature (°C), WM= minimum wind speed (km/h), WX= maximum wind. Arrow indicates end of parameters included in predictive model.

Fig. 3. Linear regression of observed vs. predicted catches of *R. dominica* per trap at grain elevators and forest validation sites for one week period in central Oklahoma. Observed trap catches were from 2 April 2005 through 2 July 2005. Note the different scales for x-axis and y-axis.

Fig. 1

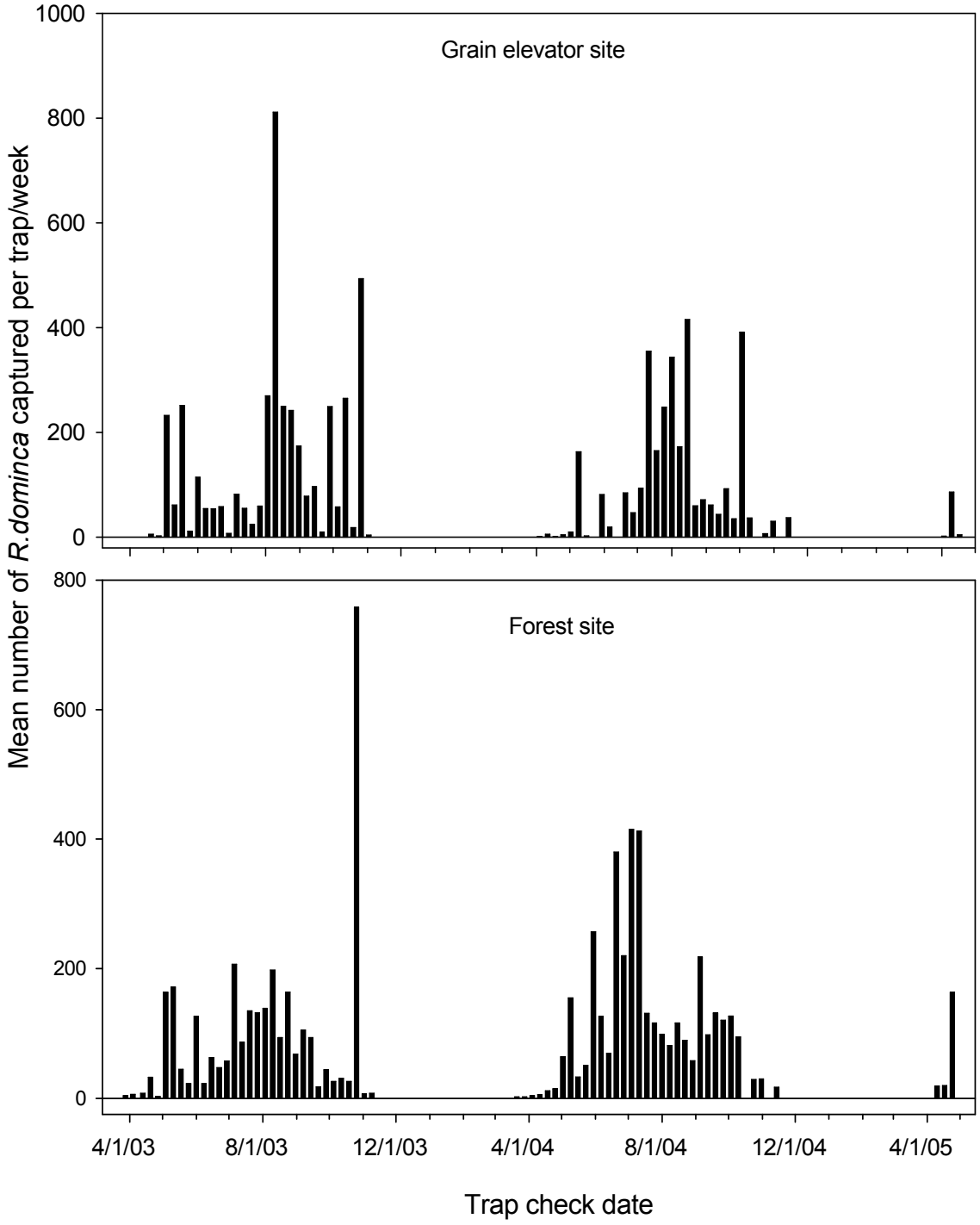

Grain elevator site
Forest site
Mean number of R.dominca captured per trap/week
1000
800
600
400
200
0
800
600
400
200
0
4/1/03
8/1/03
12/1/03
4/1/04
8/1/04
12/1/04
4/1/05
Trap check date

Fig. 2

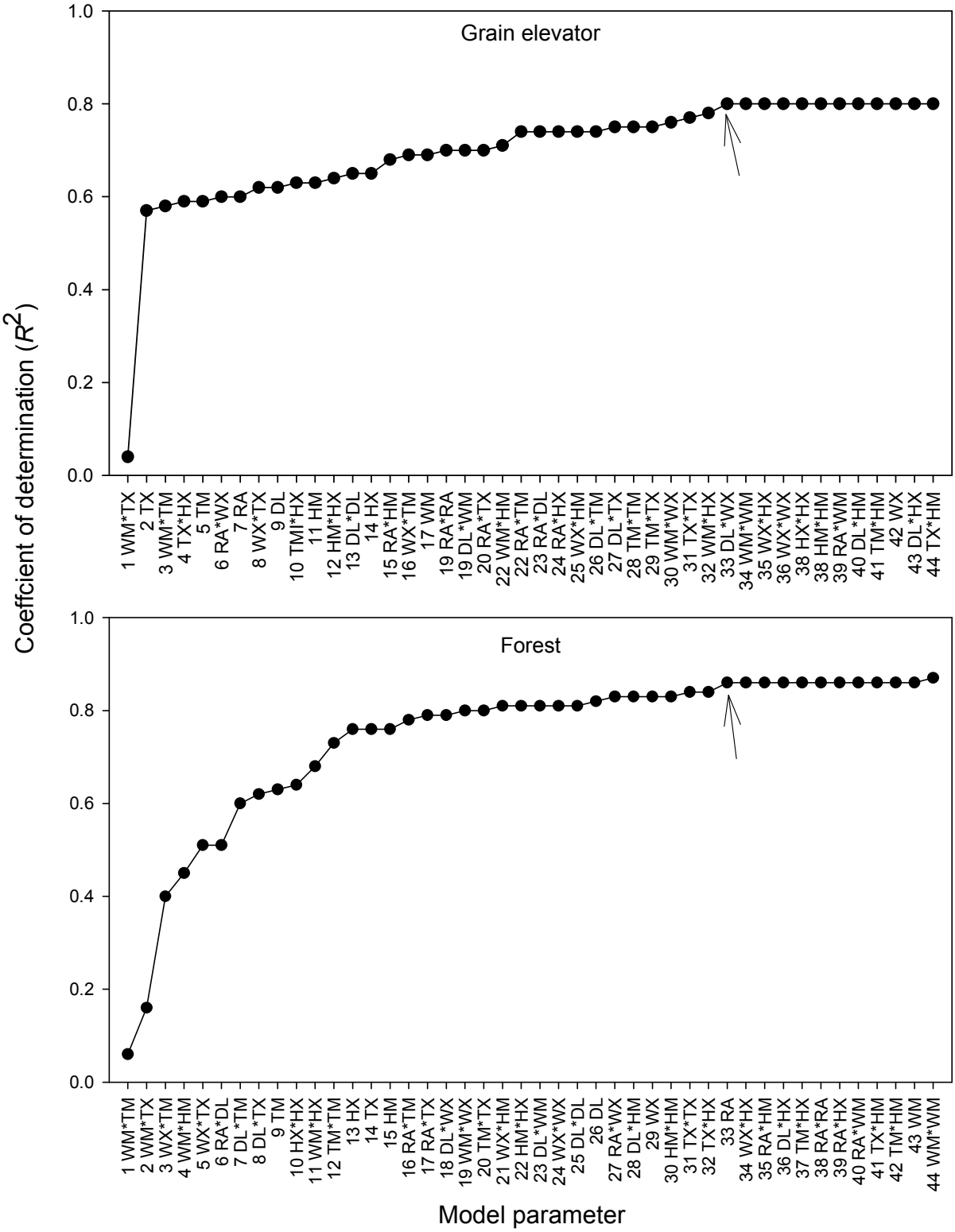
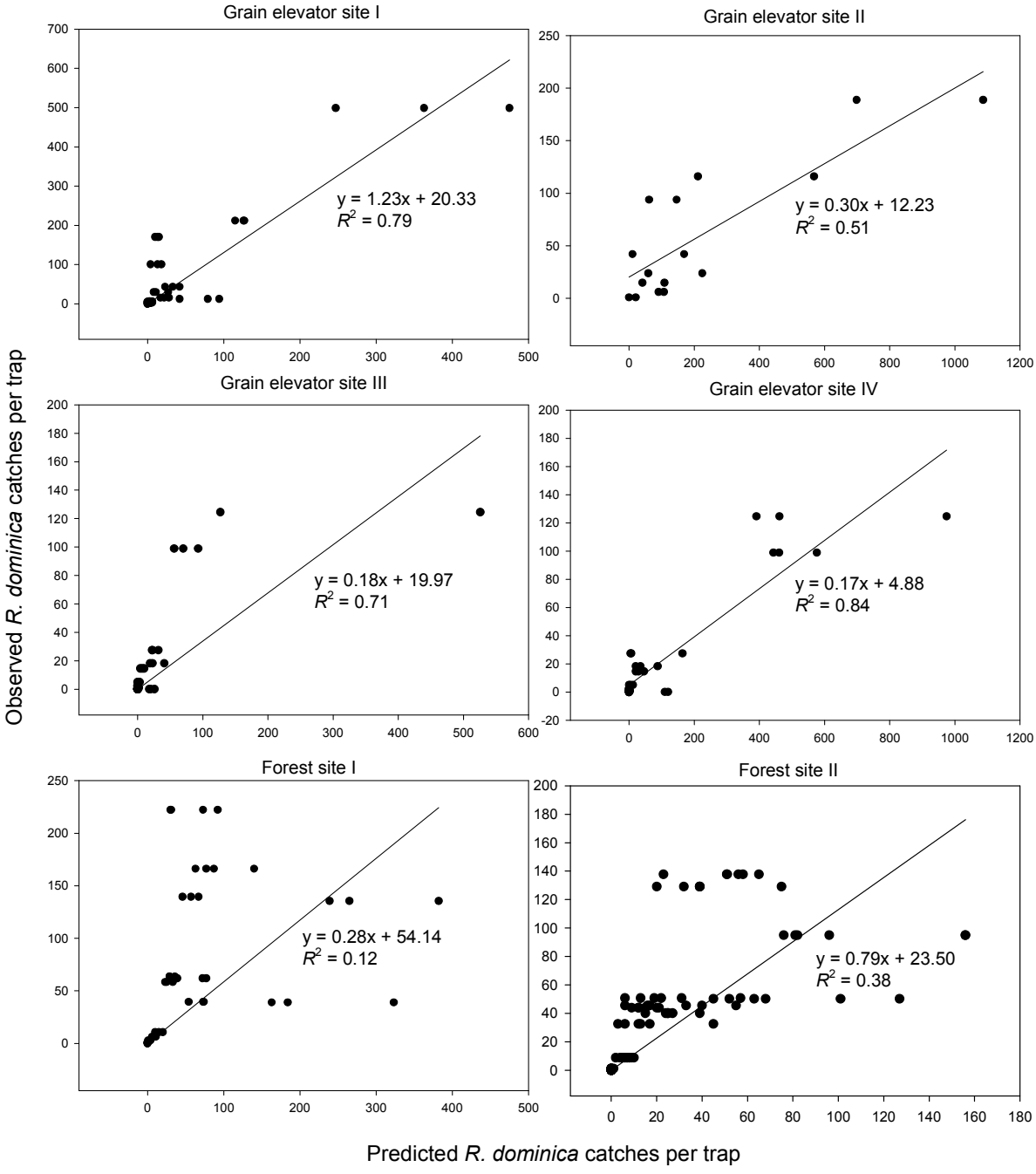

Grain elevator
Forest
Coeffcient of determination (R^2)
Model parameter
1.0
0.8
0.6
0.4
0.2
0.0
1 WM*TX
2 TX
3 WM*TM
4 TX*HX
5 TM
6 RA*WX
7 RA
8 WX*TX
9 DL
10 TM*HX
11 HM
12 HM*HX
13 DL*DL
14 HX
15 RA*HM
16 WX*TM
17 WM
19 RA*RA
19 DL*WM
20 RA*TX
22 WM*HM
22 RA*TM
23 RA*DL
24 RA*HX
25 WX*HM
26 DL*TM
27 DL*TX
28 TM*TM
29 TM*TX
30 WM*WX
31 TX*TX
32 WM*HX
33 DL*WX
34 WM*WM
35 WX*HX
36 WX*WX
38 HX*HX
38 HM*HM
39 RA*WM
40 DL*HM
41 TM*HM
42 WX
43 DL*HX
44 TX*HM
1 WM*TM
2 WM*TX
3 WX*TM
4 WM*HM
5 WX*TX
6 RA*DL
7 DL*TM
8 DL*TX
9 TM
10 HX*HX
11 WM*HX
12 TM*TM
13 HX
14 TX
15 HM
16 RA*TM
17 RA*TX
18 DL*WX
19 WM*WX
20 TM*TX
21 WX*HM
22 HM*HX
23 DL*WM
24 WX*WX
25 DL*DL
26 DL
27 RA*WX
28 DL*HM
29 WX
30 HM*HM
31 TX*TX
32 TX*HX
33 RA
34 WX*HX
35 RA*HM
36 DL*HX
37 TM*HX
38 RA*RA
39 RA*HX
40 RA*WM
41 TX*HM
42 TM*HM
43 WM
44 WM*WM

Fig. 3


Grain elevator site I
y = 1.23x + 20.33
R^2 = 0.79
Grain elevator site II
y = 0.30x + 12.23
R^2 = 0.51
Grain elevator site III
y = 0.18x + 19.97
R^2 = 0.71
Grain elevator site IV
y = 0.17x + 4.88
R^2 = 0.84
Forest site I
y = 0.28x + 54.14
R^2 = 0.12
Forest site II
y = 0.79x + 23.50
R^2 = 0.38
Observed *R. dominica* catches per trap
Predicted *R. dominica* catches per trap

CHAPTER IV

FIELD RESPONSES OF NON-TARGET SPECIES TO SEMIOCHWMICALS OF STORED-PRODUCT BOSTRICHIDAE

Accepted for publication in the Annals of the Entomological Society of America on 16 September 2005

Annals of the Entomological Society of America

Subject matter: Behavior

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Field Responses of Non-Target Species to Semiochemicals of Stored-Product Bostrichidae

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Abstract

While studying the potential occurrence of two stored grain bostrichid pests, *Rhyzopertha dominica* (F.) and *Prostephanus truncatus* (Horn), in wild habitats near Stillwater, OK using their aggregation pheromones, we observed two non-target species responding to these semiochemicals. Field experiments were conducted from 2002 to 2005 using Lindgren four-unit traps baited with either synthetic pheromone or natural semiochemicals produced by male bostrichids feeding on grain in small cages attached to traps to investigate responses of the non-target species. Ethanol was tested as a possible synergist for *R. dominica* as part of related research. *R. dominica* were commonly trapped in forested areas with its synthetic and natural pheromone, but *P. truncatus* were not captured using its natural or synthetic pheromones. Trapping results from these experiments, in conjunction with records of the known sub-tropical distribution of *P. truncatus,* led us to conclude that it probably does not occur in Stillwater, Oklahoma. However, we captured large numbers of *Zelus tetracanthus* Stål (Hemiptera: Reduviidae) males using synthetic pheromones of *R. dominica*, and this response was reduced by addition of ethanol. No *Z. tetracanthus* was caught in traps baited with natural pheromones of *R. dominica*. The results further suggest that Dominicalure-1, one of the pheromones of *R. dominica*, is attractive to *Z. tetracanthus*. Additionally, *Prostephanus punctatus* (Say), a wood boring congener of *P. truncatus*, was trapped in large numbers with natural and synthetic pheromones of *P. truncatus*. It is likely that *P. punctatus* uses the *P. truncatus* compounds Trunc-call-1 and Tunc-call-2, or similar compounds, as pheromones. Our study further revealed that Trunc-call-1 alone is attractive to *P. punctatus,* and the responses were not significantly enhanced or inhibited by the addition

of either ethanol or synthetic Trunc-call-2. Responses of *Z. tetracanthus* males to Dominicalure-1 suggest that this compound, or a structurally similar compound, plays a role in the chemical ecology of this predaceous species. Catches of *Z. tetracanthus* peaked in mid-April through May followed by a second peak in July through August. Numbers of *P. punctatus* captured in traps peaked April through May in two consecutive years.

Keywords: *Prostephanus truncatus*, *Prostephanus punctatus*, *Rhyzopertha dominica*, *Zelus tetracanthus*, Pheromones.

Introduction

Bostrichidae is one of the most destructive families of Coleoptera (Fisher 1950). Members of this family are generally wood and twig borers, but three species, *Prostephanus truncatus* (Horn), *Dinoderus bifoveolatus* (Wollaston) and *Rhyzopertha dominica* (F.) have become facultatively associated with stored cereals, dried starchy tubers and milled products (Potter 1935, Borgemeister et al. 1999). *R. dominica* is a pest of stored wheat and other small grains worldwide while *P. truncatus* and *D. bifoveolatus* are endemic to Mexico, Central America, and Africa, where they cause serious losses to stored maize and cassava (Borgemeister et al. 1999). There are reports of possible occurrence of *P. truncatus* in southern states of the United States (USA) (Gorham 1987) which raised our interest to search for this species in Oklahoma.

Early detection of pest presence is a vital component of integrated pest management programs. One way of achieving early detection is by using pheromone-baited traps. Pheromone traps provide an easy, efficient and sensitive way to detect pests and to monitor their distributions. *R. dominica* aggregation pheromones were reported by Williams et al. (1981) and Khorramshahi and Burkholder (1981) as (S)-(+)-1-methylbutyl (*E*)-2-methyl-2-pentenoate and (S)-(+)-1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate, commonly called Dominicalure-1 (DL1) and Dominicalure-2 (DL2), respectively. The pheromones of *P. truncatus* were reported (Hodges et al. 1984, Cork et al. 1991, Dendy et al. 1991) as 1-methylethyl (*E*)-2-methyl-2-pentenoate and 1-methylethyl (*E*, *E*)-2,4-dimethyl-2, 4-heptadienoate, commonly referred to as Trunc-call-1(T1) and Trunc-call-2 (T2), respectively. Similar to *R. dominica* and *P. truncatus*, male *D. bifoveolatus* produces aggregation pheromones that consists of two hydroxyl ketones, (4*R*,6*S*,7*R*)-4,6-

dimethyl-7-hydroxynonan-3-one and (3*R*,5*S*,6*R*)-3,5-dimethyl-6- hydroxynonan-2-one (Borgemeister et al. 1999, Tolasch et al. 2002). Commercially available aggregation pheromones of *P. truncatus* and *R. dominica* have been used in Africa, Latin America and the USA (Hodges 1986), and have proven to be useful for detecting and monitoring these beetles.

Unlike *P. truncatus* and *D. bifoveolatus*, *R. dominica* is not known to attack cereals in the field, but can be found infesting grain, in supposedly clean stores, within weeks or months after storage (Gates 1995, Hagstrum 2001) suggesting possible migration between potentially natural uncultivated habitats and enclosed grain storage facilities. The suggestion of possible migration from wild habitat is supported by the fact that *R. dominica* has been trapped in diverse environments, including woodlands that are substantial distance from grain storage facilities (Cogburn 1988, Edde et al. 2005). Adult *P. truncatus* have the habit of leaving granaries and boring into wood, and have been reported to bore into pine and black walnut trees (Fisher 1950). However, the connections between the wild and agricultural habitats of stored product bostrichids are yet to be clearly delineated. It is likely that wild habitats may serve as a temporary niches or alternate food sources during the absence of preferred hosts. Thus, understanding the bioecology of these species would help in formulating effective pest management strategies and in predicting the likelihood of different species occurring in stored products.

While conducting experiments with pheromone-baited traps to study the potential occurrence of *P. truncatus* and *R. dominica* in wild habitats in Stillwater, Oklahoma, we observed that two non-target species of insects were attracted to the pheromones tested.

One of the non-target species was *Prostephanus punctatus* (Say), a congener to *P. truncatus*, and it was consistently attracted to pheromones of *P. truncatus*. The second non-target species was *Zelus tetracanthus* Stål (Hemiptera: Reduviidae) a predatory assassin bug, and it was attracted to pheromones of *R. dominica*. We are unaware of any documented information on the chemical ecology of *P. punctatus* and *Z. tetracanthus*. Here we describe the experiments that were conducted with the two non-target species, and discuss the possible significance of these results.

Materials and Method

Study sites: Trapping was conducted at three locations near Stillwater, OK: Pasture II **(**36°03′N; 097°10′W, approximately 607 ha), Lake Carl Blackwell (36°07′N; 097°13′W, approximately 268 ha) and Stored Products Research and Education Center (SPREC) (36°03′N; 097°08′W). Pasture II and Lake Carl Blackwell are naturally regenerating woodlands dominated by *Quercus muhlenbergii* Engelm (Fagaceae.), *Celtis occidentalis* L. (Ulmaceae); *Quercus stellata* Wangenh (Fagaceae), *Pinus taeda* L. (Pinaceae), *Cercis canadensis* L. (Fabaceae), *Juniperus virginiana* L. (Cupressaceae), with each site at a density of about 340 trees per ha. SPREC is a training and grain storage facility having 58 steel bins with combined capacity of 1,143 metric tons of grains, but holding approximately 327 metric tons of hard red winter wheat *Tritichum aestivum* (L.) during most of the experimental period. The perimeter of SPREC has patches of trees concentrated to the north (about 2.5 km) and to the south (about 1.5 km) of the facility, and the site is essentially without trees.

Traps: Lindgren four-unit funnel traps (Phero Tech, Delta, British Columbia, Canada) were used in the experiments. Traps were hung on polyvinyl chloride (PVC) tubing within 1.7 m of the ground. Traps were spaced at least 20 m apart. Collection cups contained pieces of No-Pest® strip (United Industries Corp., St. Louis, MO) releasing dichlorvos to kill captured insects.

Lures: With the exception of the commercially fabricated *P. truncatus* pheromones Bullet Lure® (Insects Limited, Inc. Westfield, IN, USA), *P. truncatus* and *R. dominica* lures used in the studies were fabricated in our laboratory. Number 11.5 sleeve stoppers (Fisher Scientific, Pittsburgh, PA; referred to here as rubber septa) were first cleaned by soaking overnight in dichloromethane and then allowed to air dry under a fume hood for 24 h. Pheromones were applied to the interior surface of rubber septa as a 50% hexane solution containing mixtures of DL-1 and DL-2 or T1 and T2. Another set of lures was prepared by applying the individual components DL-1, DL-2, T1 or T2 to rubber septa. Treated septa were dried in the fume hood 1 h to allow the hexane to evaporate. The chemical purity of DL-1 and DL-2 was 96 and 94%, respectively, as determined by our laboratory using gas chromatography; however, we do not have information on the chemical purity of T1 or T2 used in the study. Ethanol was tested because we were originally interested in its role in the response of *R. dominica* to pheromones in other research (not elaborated here). Vapors from ethanol (95% USP grade, Pharmco, Brookfield, CT) were released from a 250 ml screw-cap plastic bottle, with 4 cm of a 15-cm cotton dental wick protruding from a hole cut in the center of the screw cap. Bottles were hung on the lowest funnel of the trap. Bottles were replaced weekly with fresh ethanol and re-randomized by moving traps and bottles within a site to minimize bias in

trap capture due to trap location.

Experiment 1. Traps were deployed weekly in Pasture II from 10 April through 3 June 2003 to study responses of *Z. tetracanthus* to synthetic *R. dominica* pheromones and ethanol. Treatments were pheromones (a mixed solution of 5 mg of DL-1 and 5 mg of DL-2 applied to single septum) alone, pheromone plus ethanol, ethanol alone, and unbaited traps (control). One pheromone-impregnated rubber septum was used per trap and was hung on the second funnel from the bottom of the trap. *Z. tetracanthus* captured were sorted by sex and counted. Sexes were determined according to Hart (1986). The study was conducted as a randomized complete block design in which blocking was by time (week). There were seven blocks containing two replications of each treatment.

Experiment 2. Based on the observed responses of *Z. tetracanthus* to *R. dominica* pheromone, we conducted an experiment from 13 May to 10 June 2005 to determine which of the two *R. dominica* pheromones were attractive to Z. *tetracanthus*. As with other stored product Bostrichidae pests (Borgemeister et al. 1999), feeding is required for pheromone production by male *R. dominica* (Mayhew and Phillips 1994). Therefore, in the same experiment we tested the response of *Z. tetracanthus* to naturally-produced pheromones. Ten laboratory-reared male *R. dominica* (about four weeks old from laboratory colonies maintained on wheat and derived from local field populations over one year prior to tests) were placed on 10 g of wheat seeds at 12-14% moisture in cylindrical tubes (30 cm long, 2 cm dia.) made of copper wire screen mesh that were plugged at both ends with rubber stoppers. The sex of beetles was determined by gently squeezing the last three ventral abdominal segments to extrude the genitalia. Treatments with synthetic pheromones were traps baited with a septum dosed with 10 mg of DL-1,

traps with septa containing 10 mg of DL-2 and traps baited with a septum containing the mixture of 5 mg DL-1 plus 5 mg DL-2. Two additional treatments consisted of a tube with wheat seeds alone and control traps without attractant. The study was conducted as a randomized complete block design in which there were five blocks (weeks) containing two replications of each treatment.

Experiment 3. Traps were deployed weekly at Lake Carl Blackwell from 2 May through 4 June 2003 to study responses of *P. punctatus* to natural and synthetic components of *P. truncatus* aggregation pheromones. The synthetic *P. truncatus* aggregation pheromones T1 and T2 were released together from a Bullet Lure® (loading rates and release rates unknown) hung on the second funnel from the bottom of the trap. As noted earlier, feeding is required for pheromone production in *P. truncatus* (Scholz et al. 1997). Thus, to test the response of P. *punctatus* to naturally produced pheromone, we placed ten laboratory-reared male *P. truncatus* (about one week old) on 10 g of corn (*Zea mais* L.) seeds at 12-13% moisture in tubes similar to those used in the *Z. tetracanthus* study above. The sex of beetles was determined as described for *R. dominica*. Two additional treatments were tube with maize seeds alone and control traps without attractant. *P. truncatus* used in the study originated from Benin Republic (West Africa) and have been maintained on corn seeds in our laboratory since 1999. Captured *P. punctatus* were sorted by sex and counted on a weekly basis. The study was conducted as a randomized complete block design in which there were four blocks (weeks) containing two replications of each treatment.

Experiment 4. Based on observations in Experiment 3, we conducted additional studies to determine which of the two *P. truncatus* pheromones were attractive to *P. punctatus*.

Traps baited with septa containing either 10 mg of T1, 10 mg of T2, or a mixture of 5 mg of T1and 5 mg of T2 were deployed for four and five weeks at Pasture II and Lake Carl Blackwell, respectively, from 15 June 2004 to 16 July 2004. Additional treatments included combinations of individual pheromone components or the T1/T2 mixture with ethanol. Traps without attractant served as controls. Captured *P. punctatus* were sorted by sexes and counted. The sex of *P. punctatus* was determined as described for *P. truncatus.* The study was conducted as a completely randomized block design in which blocking was by location. There were two blocks per study site, and each treatment was tested for four weeks at both sites, and for an additional week in one location at one of the sites.

Experiments 5 and 6. Seasonal activity of male *Z. tetracanthus* was monitored continuously in Experiment 5 from March 2003 through December 2004 at Pasture II using two traps baited with *R. dominica* pheromones (mixture of 5 mg of DL1 and 5 mg of DL2). In Experiment 6 the seasonal activity of *P. punctatus* was documented at SPREC from March through December 2004 and at Lake Carl Blackwell from March 2004 through 11 June 2005 using traps baited with Bullet Lures® containing the two *P. truncatus* pheromones. Traps were emptied, re-baited and counts collated on a weekly basis in both studies. Mean weekly air temperature data for the study locations were obtained from weather stations located within 3 km of the trapping sites (http://www.mesonet.org). Data on weekly trap catches of *Z. tetracanthus* and *P. punctatus* were expressed as a proportion of total trap capture and plotted against time of captures to show seasonal flight pattern of the species.

Data Analysis. Data (experiments 1- 4) were analyzed using SAS Proc Mixed Procedures ($P< 0.05$) (SAS Institute 2001). Locations and weeks were considered as

random effects in the respective mixed models and therefore included in the RANDOM statement within the PROC MIXED code. Prior to data analysis, count data were transformed using Log (X +1) to satisfy the assumptions of normality and homogeneity of variance. Actual means and standard errors are presented in the figures. Means were compared using Tukey's Studentized range test (Tukey 1953). A Chi-square analysis was used to test if the sex ratio of *P. punctatus* captured differed significantly from 1:1.

Results and Discussion

Only male *Z. tetracanthus* were captured in our experiments. In Experiment 1 the unbaited traps and those baited with wheat or ethanol alone did not capture any *Z. tetracanthus*; since these treatments have no variability they were not included in the data analysis to avoid interference with the assumption of homogeneity of variances in the model (Reeve and Strom 2004). The greatest numbers of Z. *tetracanthus* were caught in traps baited with the lure containing a mixture of synthetic pheromones, and this was significantly different from traps baited with ethanol and the synthetic *R. dominica* pheromones, which caught significantly fewer insects (Fig. 1).

Results from Experiment 2 confirmed the attractiveness of *R. dominica* pheromones to *Z. tetracanthus,* and provided three other interesting observations. First, DL-1 was the most attractive compound to the *Z. Tetracanthus* males, accounting for about 68% of the total number of *Z. Tetracanthus* captured. Second, the number of *Z. tetracanthus* captured in traps baited with DL-1 alone was more than double the number of insects caught in traps baited with a combination of DL-1 and DL-2; however, the difference was not significantly different (t-test = -0.60; df = 22; $P = 0.56$) (Fig. 2). DL-2 alone captured less

than 3% of the total trap catches. Therefore, DL-1 or a compound similar to it most likely plays a role in the chemical ecology of this predaceous species, and DL-2 is probably inactive and may even suppress response of *Z. tetracanthus* to DL-1. The third observation was that no *Z. tetracanthus* was attracted to traps baited with live *R. dominica* releasing natural pheromone. The fact that we captured large numbers of *R. dominica* in traps baited with live beetles and in traps baited with synthetic pheromone (e.g., an average of 12.1± 9.8 *R. dominica* per trap per week in traps baited with males, and an average of 104.5 ± 90.7 to traps baited with the synthetic pheromone mixture), but not a single *R. dominica* in traps baited with wheat alone or unbaited traps, confirmed the release of pheromone by the males on wheat. Many volatile compounds in addition to DL-1 and DL-2 are produced by *R. dominica* (Seitz and Ram 2004), and it is likely; therefore, that one or more of these compounds could have suppressed responses of *Z. tetracanthus* to the DL-1 from traps baited with live male *R. dominica*.

Little is known on the biology of *Z. tetracanthus*. The insects are medium to large assassin bugs, approximately 13-15 mm in length, with a wide range of known prey, including cotton fleahoppers *Pseudatomoscelis seriatus* (Reuter), *Lygus* bugs, aphids, pink bollworm *Pectinophora gossypiella* (Saunders) larvae, cotton bollworm, *Helicoverpa zea* (Boddie), larvae and tobacco budworm, *Heliothis virescens* (Fab.), larvae (Frank and Slosser 1996). *Z. tetracanthus* are known to be one of the few predators that have the ability to feed on boll weevil adults and mature bollworm larvae (Frank and Slosser 1996). We are unaware of any reports regarding the chemical ecology of *Z. tetracanthus*. However, several pheromones of other hemipteran species have been documented, including sex, courtship, attractant, aggregation, and alarm

pheromones (Aldrich 1988, Drijfhout and Groot 2001). In insects, aggregation pheromones are produced to congregate conspecific individuals for feeding or reproduction, and alarm pheromones serve to rapidly disperse a group, usually as a response to predation (Regnier and Law 1968). Sex pheromones may be liberated for the purpose of locating the emitter and subsequent mating, and attractant pheromones are suggested to be prerequisites for successful courtship and mating among several species of insects (Baker 1989, Gillot 1995). Unlike aggregation and alarm pheromone, which may be produced by either males or females of the same species, sex and attractant pheromones are usually sex-specific in their production and response. Thus, the apparent attraction of only males to semiochemical-baited traps observed in our study further suggests that female *Z. tetracanthus* may be producing and using a compound or compounds the same or similar to DL-1 produced by *R. dominica* as chemical cues to recruit potential mates or as a means of achieving successful courtship and mating with males. This phenomenon of using sex-specific pheromones for mate recruitment and courtship has been suggested in other Hemiptera such as the rice leaf bug, *Trigonotylus caelestialium* (Kirkaldy) (Kakizaki and Sugie 2001) and *Lygocoris pabulinus* (L.) (Groot et al. 1999).

Male *Z. tetracanthus* may also be responding kairomonally to DL-1 or similar compounds as cues for prey location. The restriction to males only in this scenario of kairomonal response could suggest that males locate prey and then call females for mating and possibly feeding on prey. However, we are unaware of research about prey-mediated mate-finding behavior in predaceous Reduviidae, so our conjecture here has no documented support from previous work on this or other Reduviidae. *Z. tetracanthus*

is an important predator of a wide range of cotton pests (Frank and Slosser, 1996); thus information on chemical ecology of this bug could enhance their utility as biological control agents or represent an additional component of an integrated pest management system if semiochemicals were used to exploit recruitment and mating of assassin bugs in prey habitats.

The inhibitory or interruptive effect of ethanol on the pheromonal response by *Z. tetracanthus* observed in the present study is contrary to the attractive or synergistic enhancing effects on trap catches that have been associated with combinations of ethanol plus other semiochemicals for some tree-infesting Coleoptera species (Phillips et al. 1988, Czokajlo and Teale 1999) and in *R. dominica* (unpublished data), but is in agreement with earlier observations by Byers et al. (1998) and Byers et al. (2000). These latter workers observed reduced trap capture when ethanol was combined at a relatively high rate with synthetic pheromone in some coleopteran beetles in the family Scolytidae such as *Pityogenes bidentatus* (Herbst) and *Pityogenes quadridens* (Hartig). Ethanol is a common respiratory by-product associated with stressed trees or dying plant materials (Joseph et al. 2001), and thus it seems logical for it to affect behavior of insects such as bark and ambrosia beetles that colonize such materials. The aversion of *Z. tetracanthus* to ethanol may suggest that stressed or decomposing plants did not represent a suitable habitat for this species. The rate of release of ethanol vapors is known to have an effect on the response of some insect species to ethanol or combinations of pheromone plus ethanol (Phillips et al. 1988, Byers 1992, Byers et al. 2000, Joseph et al. 2001) and it is possible that a lower release rate might have yielded different results in our experiments, but this factor was not considered in the present

study.

Our results on seasonal captures of *Z. tetracanthus* in pheromone-baited traps allow us to suggest two peaks of flight activity for this species in Stillwater, OK (Fig. 3). The main peak occurs from mid-April through May followed by a second peak in July through August. No *Z. tetracanthus* were captured from September through March. There was year-to-year variation in capture of *Z. tetracanthus* (Fig. 3); much of which is likely due to differences in survival rate and weather differences between years (Allsopp and Logan 1999). Most *Z. tetracanthus* were caught during weeks when mean weekly temperatures were 15^0 C.

No larger grain borers, *P. truncatus*, were captured in experiment 3 in which synthetic and natural pheromones of *P. truncatus* were deployed. This observation, in conjunction with the known sub-tropical distribution of this species (Hodges 1986), suggests that *P. truncatus* probably does not occur in Stillwater, Oklahoma. However, substantial numbers of the congener *P. punctatus* were captured in this experiment. *P. punctatus* closely resembles *P. truncatus*, but can be separated from the latter by its larger size (4-5 mm long, 1.5-1.7 mm width) and the presence of one or two distinct tubercles on the distal tip of each elytron (Fisher 1950). Most beetles captured in our study had two pairs of tubercles on each elytron, but the inner pair is often smaller or hardly seen. *P. punctatus* has been suggested as a potential pest of oak and pecan (Fisher 1950). Both male and female *P. punctatus* were captured, but females accounted for about 65% of total beetles sexed. A Chi-square analysis confirmed that the sex ratio differed significantly from 1:1 ($F = 21.35$; df = 1; $P < 0.001$). Traps baited with synthetic *P. truncatus* pheromone captured five times as many *P. punctatus* as did traps baited with

male *P. truncatus* feeding on maize and producing natural pheromone, while no *P. punctatus* was attracted to maize alone or to unbaited traps (Fig. 4). Pheromone release rate in male *P. truncatus* feeding on maize seeds averaged 2.4 µg per day in a laboratory study (Hodges et al. 2002). Therefore, we may assume at best, that the 10 beetles released approximately 168 µg of pheromones per week. We assume that the Bullet Lure® release synthetic pheromones at a much higher and more constant rate than live males. The presumed higher release rates of the synthetic compounds relative to those released by male beetles over the same period may have contributed to higher trap catches compared to synthetic pheromones (Dendy et al. 1991, Scholz et al. 1997). Furthermore, pheromone release in most insects is not a continuous process, but follows a diel rhythmic pattern (Carde and Elkinton 1984, Rafaeli and Gileadi 1995). Therefore, much of the variation in trap captures between natural and synthetic baited traps may be linked to higher dosage and/or uninterrupted emission of pheromone plumes from traps baited with the synthetic compounds, which likely enhanced the effective capture area or lengthened the duration of traps attractiveness. Our findings correspond to those by Scholz et al. (1997) on *P. truncatus*. These workers observed a 13-fold increase in the number of beetles captured in traps baited with 2 mg of the synthetic compound than in traps with maize cob baited with one male.

The results of experiment 4 confirmed the attractiveness of *P. truncatus* pheromones to *P. punctatus* observed in experiment 2 (Fig. 5). T1 was the most attractive *P. truncatus* pheromone for *P. punctatus*; T2 by itself captured few beetles, and combining T1 with T2 and/or ethanol did not significantly enhance or inhibit responses by *P. punctatus*. In general, therefore, it seems that *P. punctatu*s likely uses components

identical to those of *P. truncatus*, particularly T1, as aggregation pheromones, and we suspect that males produce the pheromones because, as with other bostrichids, both male and female *P. punctatus* were attracted to *P. truncatus* pheromones, but responses were skewed toward females. Interspecific cross-attraction has been suggested among the Bostrichidae (Borgemeister et al. 1999), and it is known in many other families of Coleoptera (Lainer and Burkholder 1974). If *P. truncatus* and *P. punctatus* are allopatric and do not co-occur in the same habitats or geographic regions, then use of identical pheromones by both species would not pose a problem for reproductive isolation or mistaken orientation in mate-finding, such as that documented for Scolytidae in the genus *Ips* (Lanier and Wood 1975). Alternatively, *P. punctatus* may not use these or similar chemicals for its own pheromones, but it may simply respond exploitatively to pheromones of other Bostrichidae as signals for the location of suitable host material. In our study, the lack of response by *P. punctatus* to ethanol vapor may be a consequence of this species not inhabiting stressed or recently dead roots or stumps. Saproxylic species attack or breed in stressed or newly dead trees and are known to use ethanol and other products associated with the microbial degradation in host recognition; some of these species are attracted to ethanol alone (Joseph et al. 2001).

Our data on long term monitoring of *P. punctatus* traps in a forested habitat and near a grain storage facility are shown in Figure 6. The perimeter of the grain storage facility had patches of trees concentrated to the north (about 2.5 km) and to the south (about 1.5 km) of the facility. Therefore, *P. punctatus* captured near the grain storage site is indicative of a strong flying ability and potential by the beetle to migrate into a grain storage facility infested by *P. truncatus*, if such an opportunity existed. Adult *P.*

punctatus commence flight early in spring, prior to most tree leaf growth. Our data suggests a single peak period of flight activity occurring in April through May when the mean weekly temperature is 10 to 15°C. No *P. punctatus* were captured from September through December.

Preliminary attempts to rear *P. punctatus* on plant species in the laboratory suggest this species is able to feed and marginally reproduce on corn seeds, *Zea mais* (L.) and dried cassava tubers, *Manihot esculenta* Crantz. (Edde unpublished data). The ability to reproduce on corn seeds and cassava tubers is an indication that *P. punctatus* has the potential to become an agricultural pest. Many of the present-day stored product insect pests, including Bostrichidae, were undoubtedly from wood boring ancestors and have undergone behavioral changes in food habits, and probably underwent adjustments and adaptations to a new environment (Linsley, 1944).

Acknowledgements

We thank Edmond Bonjour, Henry Fadamiro, Kris Giles and Phil Mulder for reviewing initial draft of the manuscript. We are also grateful to Don Arnold for assistance with identification of *Z. tetracanthus* and *P. truncatus*. Phillip Taylor helped with identification of trees at the experimental sites. James Barrett Robertson assisted with counting of insects. Wendell Burkholder, USDA, Madison, WI (retired), and Alain Van Rhyckenham, Insects Limited Inc., Westfield, IN, graciously supplied *R. dominica* and *P. truncatus* pheromones, respectively. The USDA CSREES (Risk Avoidance and Mitigation Program) under Agreement No 2000-05385 funded this project.

References cited

Aldrich, J. R. 1988. Chemical ecology of the Heteroptera. Ann. Rev. Entomol. 33: 211-238.

Allsopp, P. G., and D. P. Logan. 1999. Seasonal flight activity of scarab beetles (Coleoptera: Scarabaeidae) associated with sugarcane in southern Queensland. Aus. J. Entomol. 38: 219-226.

Baker, T. C. 1989. Sex pheromone communication in Lepidoptera: New research progress. Experientia 45: 248-262.

Borgemeister, C., K. Schaefer, G. Goergen, S. Awande, M. Setamou, and H. -M. Poehling. 1999. Host-finding behavior of *Dinoderus bifoveolatus* (Coleoptera: Bostrichidae), an important pest of stored cassava: The role of plant volatiles and odors of conspecifics. Ann. Entomol. Soc. Am. 92: 766-771.

Byers, J. A. 1992. Attraction of bark beetles *Tomicus piniperda*, *Hylurgus palliates*, and *Trypodendron domesticus* and other insects to short-chain alcohols and monoterpenes. J. Chem. Ecol. 18: 2385-2402.

Byers, J. A., Q. H. Zhang, and G. Birgersson. 2000. Strategies of a bark beetle, *Pityogenes bidentatus*, in an olfactory landscape. Naturwissenschaften 87: 503-507.

Byers, J. A., Q. H. Zhang, F. Schlyter, and G. Birgersson. 1998. Volatiles from nonhost birch trees inhibit pheromone response in spruce bark beetles. Naturwissenschaften 85: 557-561.

Carde, R. T., and J. S. Elkinton. **1984**. Field trapping with attractants: Methods and interpretation, pp. 111-129. *In* H. E. Hummel, and T. A. Miller (eds.), Techniques in pheromone research. Springer-Verlag, New York.

Cogburn, R. R. 1988. Detection, distribution and seasonal abundance of *Sitotroga cerealella* and *Rhyzopertha dominica* as indicated by pheromone-baited adhesive traps, p 451. *In* Proceedings, XVIII International Congress of Entomology**,** University of British Columbia, 3-9 July 1988**,** Vancouver. B. C. Canada.

Cork, A., D. R. Hall, R. J. Hodges, and J. A. Pickett. 1991. Identification of major components of the male produced aggregation pheromones of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). J. Chem. Ecol. 17: 789-803.

Czokajlo, D., and S. A. Teale. 1999. Synergistic effect of ethanol to alpha-pinene in primary attraction of the larger pine shoot beetle, *Tomicus piniperda***.** J. Chem. Ecol. 25: 1121-1130

Dendy, J., P. Dobie, J. A. Smith, and J. L. Urono. 1991. Trials to assess the effectiveness of new synthetic pheromone mixtures for trapping *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in maize stores. J. Stored Prod. Res. 27: 69-74.

Drijfhout, F. P., and A. T. Groot. 2001. Close-range attraction in *Lygocoris pabulinus* (L.). J. Chem. Ecol. 27: 1133-1149.

Edde, P. A., T. W. Phillips, and M. D. Toews. 2005. Responses of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromones as influenced by trap design, trap height and habitat. Environ. Entomol. (in press).

Fisher, W. S. 1950. A revision of the North American species of beetles belonging to the family Bostrichidae. Miscellaneous Publication No. 698. U.S. Department of Agriculture, Washington, DC.

Frank, W. A., and J. E. Slosser. 1996. An illustrated guide to the predaceous insects of the Northern Texas Rolling Plains. The Texas Agricultural Experiment Station MP-1718. The Texas A&M University, College Station, Texas.

Gates, M.W. 1995. Population dynamics of lesser grain borer, rusty grain beetle, and *Cephalonomia waterstoni* in commercial elevators. M.S. thesis, Oklahoma State University Stillwater, Oklahoma

Gillot, C. 1995. Entomology, 2nd ed. Plenum Press, NY.

Gorham, J. R . 1987. Insects and mites in food: an illustrated key. Agricultural Handbook Number 655. U.S. Department of Agriculture, Washington, DC.

Groot, A. T., R. Timmer, G. Gort, G. P. Lelyveld, F. P. Drijfhout, T. A. Van Beek, and J. Hans Visser. 1999. Sex-related perception of insect and plant volatiles in *Lygocoris pabulinus.* J. Chem. Ecol. 25: 2357 – 2371.

Hagstrum, D. W. 2001. Immigration of insects into bins storing newly harvested wheat on 12 Kansas farms. J. Stored Prod. Res. 37: 221-229.

Hart, E. R. 1986. Genus *Zelus* Fabricius in the United State, Canada, and Northern Mexico (Hemipteran: Reduviidae). Ann. Entomol. Soc. Am. 79: 535-548.

Hodges, R. J., A. Cork, and D. R. Hall. 1984. Aggregation pheromones for monitoring the greater grain borer *Prostephanus truncatus*, pp. 255-260. *In* Proceedings British Crop Protection Conference. Pest and Diseases. November 1984, Brighton, UK.

Hodges, R. J. 1986. The biology and control of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) – a destructive storage pest with an increasing range. J. Stored Prod. Res. 22: 43-48.

Hodges, R. J, L. A. Birkinshaw, D. I. Farman, and D. R. Hall. 2002. Intermale variation in aggregation pheromone release in *Prostephanus truncatus*. J. Chem. Ecol. 28: 1665-1674.

Joseph, G., R. G. Kelsy, R. W. Peck, and C. G. Niwa. 2001. Response of some scolytids and the predators to ethanol and 4-Allylanisole in pine forest of central Oregon. J. Chem. Ecol. 27: 697-715.

Kakizaki, M., and H. Sugie. 2001. Identification of female sex pheromone of the rice leaf bug, *Trigonotylus caelestialium*. J. Chem. Ecol. 27**:** 2447-2458.

Khorramshahi, A. and W. E. Burkholder. 1981. Behavior of the lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Male-produced aggregation pheromone attracts both sexes. J. Chem. Ecol. 7: 33-38.

Lanier, G. N., and W. E. Burkholder. 1974. Pheromone in speciation of Coleoptera. pp. 161-199. *In* M. C. Birch (eds.), Pheromones. North-Holland, New York.

Lanier, G. N., and D. L. Wood. 1975. Specificity of response to pheromones in the genus *Ips* (Coleoptera: Scolytidae). J. Chem. Ecol. 1: 9-23.

Linsley, G. E. 1944. Natural sources, habitat, and reservoirs of insects associated with stored food products. Hilgardia 16: 187-225.

Mayhew, T. J. and T. W. Phillips. 1994. Pheromone biology of the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae), pp. 541-544. *In* Proceedings, 6th International Working Conference on Stored-Product Protection, 17-23 April 1994, Canberra, Australia, CAB International, Wallingford, UK.

Phillips, T.W., A. J. Wilkening, T. H. Atkinson, J. L. Nation, R. C. Wilkinson, and J. L. Foltz. 1988. Synergism of turpentine and ethanol for certain pine-infesting beetles (Coleoptera). Environ. Entomol. 17: 456-462.

Potter, C. 1935. The biology and distribution of *Rhyzopertha dominica* (Fab.). Trans. Royal Entomol. Soc. 83: 449-482.

Rafaeli, A. and C. Gileadi. 1995. Factors affecting pheromone production in the stored product moth, *Plodia interpunctella*: a preliminary study. J. Stored Prod. Res. 31: 243-247.

Reeve, J. D., and B. L. Strom. 2004. Statistical problems encountered in trapping studies of scolytids and associated insects. J. Chem. Ecol. 30: 1575-1590.

Regnier, F. E., and J. H. Law. 1968. Insect pheromones. J. Lipid Res. 9: 541-551.

SAS Institute. 2001. SAS/STAT User's Guide, version 8, SAS Institute Inc., Cary, NC

Scholz, D., C. Borgemeister, W. G. Meikle, R. H. Markham, and H. M. Poehling. 1997. Infestation of maize by *Prostephanus truncatus* initiated by male-produced pheromone. Ent. Exp. Appli. 83: 53-61.

Seitz, L. M., and M. S. Ram. 2004. Metabolites of lesser grain borer in grains. J. Agric. Food Chem. 53: 898-908.

Tolasch, T., C. Borgemeister, Y. Masuda, K. Fujita, K. Mori, and W. Francke. 2002. The aggregation pheromone of *Dinoderus bifoveolatus* Wollaston (Coleoptera: Bostrichidae), p. 148. *In* Proceedings, 19th annual meeting International Society of Chemical Ecology 3-7 August 2002, University of Hamburg, Germany.

Tukey, J.W. 1953. The problem of multiple comparisons. Department of Statistics, Princeton University. Princeton, NJ.

Williams, H. J., R. M. Silverstein, W. E. Burkholder, and A. Khorramshahi. 1981. Dominicalure 1 and 2: components of aggregation pheromone from male lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). J. Chem. Ecol. 7: 759-781.

Figure legends

Fig. 1. Mean (+SE) numbers of *Z. tetracanthus* captured weekly per trap baited with synthetic *R. dominica* aggregation pheromones and ethanol in Experiment 1 in a forested habitat near Stillwater, OK from April 18 through May 30, 2003. All Z. *tetracanthus* captured were males. Lure = a synthetic mixture *R. dominica* aggregation pheromones DL-1 and DL-2. Ethanol alone or unbaited traps caught no insects, thus were deleted from the comparison. N = number of replications. Bars with the same letter above them are not significantly different (α= 0.05).

Fig. 2. Responses of *Z. tetracanthus* to natural and synthetic *R. dominica* aggregation pheromones in Experiment 2; DL-1 = Dominicalure-1 (synthetic) and DL-2 = Dominicalure-2 (synthetic). Wheat alone and unbaited traps caught no insect, thus were deleted from the comparison. N = number of replications. Bars with the same letter above them are not significantly different (α= 0.05).

Fig. 3. Proportions of Z. *tetracanthus* caught in traps baited with synthetic *R. dominica* pheromones in a forested habitat near Stillwater, OK during 2003-2004 in Experiment 5.

Fig. 4. Responses of *P. punctatus* to natural and synthetic *P. truncatus* aggregation pheromones in Experiment 3. The sexes of *P. punctatus* were captured in the estimated proportions of 65% female and 35% male (n = 345 insects determined). SYNT LURE = Synthetic *P. truncatus* aggregation pheromones (Trunc-call 1 + Trunc-call 2) released from a Bullet Lure, and MZ + PT = Male *P. truncatus* feeding on corn. Corn alone and

unbaited traps caught no *P. punctatus*, thus were deleted from the comparison. N = number of replications. Bars with the same letter above them are not significantly different (α= 0.05).

Fig. 5. Mean (+SE) numbers of *Prostephanus punctatus* attracted to components of the aggregation pheromones of *P. truncatus* and ethanol in a wooded habitat in Experiment 4. T1= Trun-call-1, T2 = Trun-call-2. Ethanol and unbaited traps caught no *P. punctatus*, thus were deleted from the comparison. N = number of replications. Bars with the same letter above them are not significantly different (α= 0.05).

Fig. 6. Pattern of occurrence of *P. punctatus* in traps baited with *P. truncatus* pheromones in a forested habitat and near a grain elevator in Stillwater, OK from 2004 to 2005 in Experiment 6.

Fig. **1**

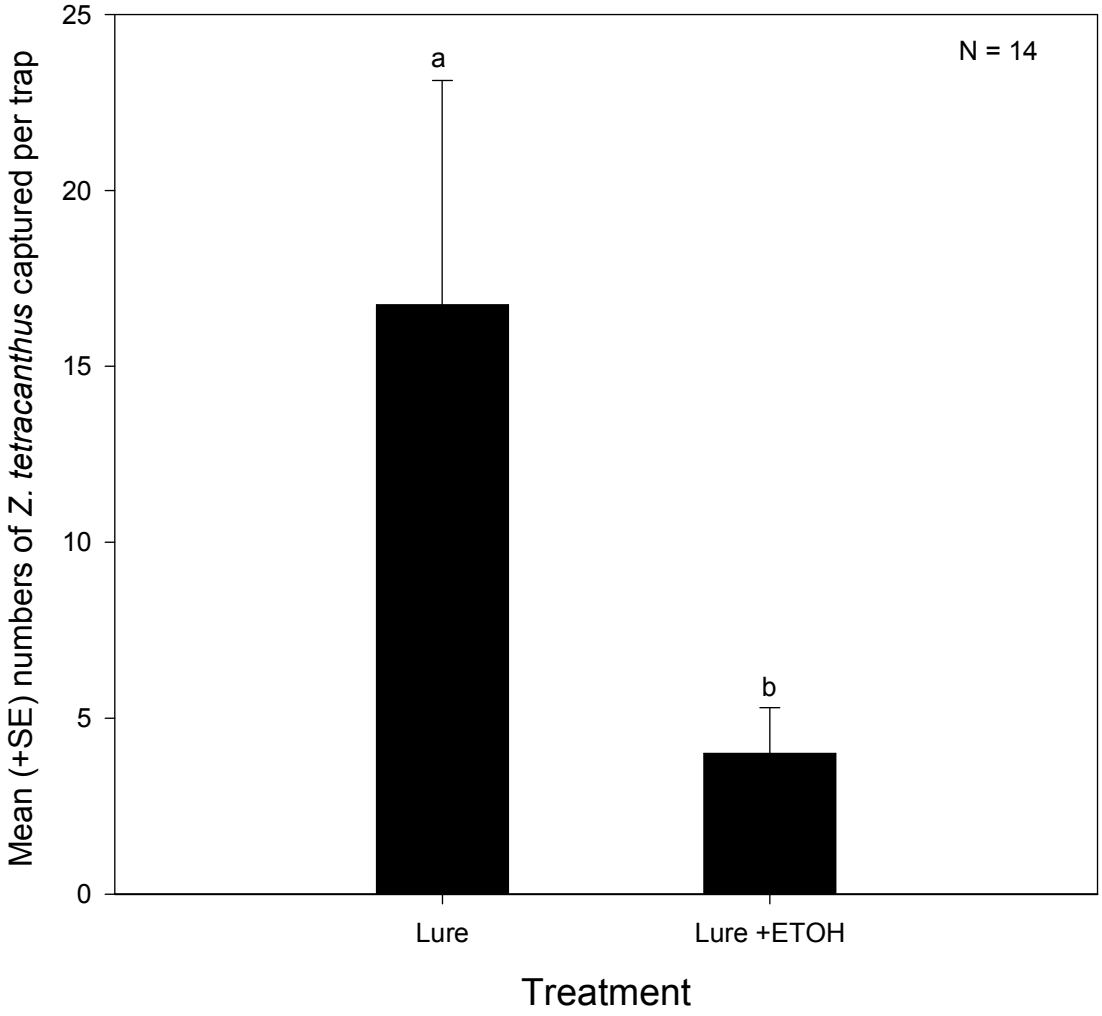

25
20
15
10
5
0
Mean (+SE) numbers of Z. tetracanthus captured per trap
a
b
N = 14
Lure
Lure +ETOH
Treatment

Fig. 2

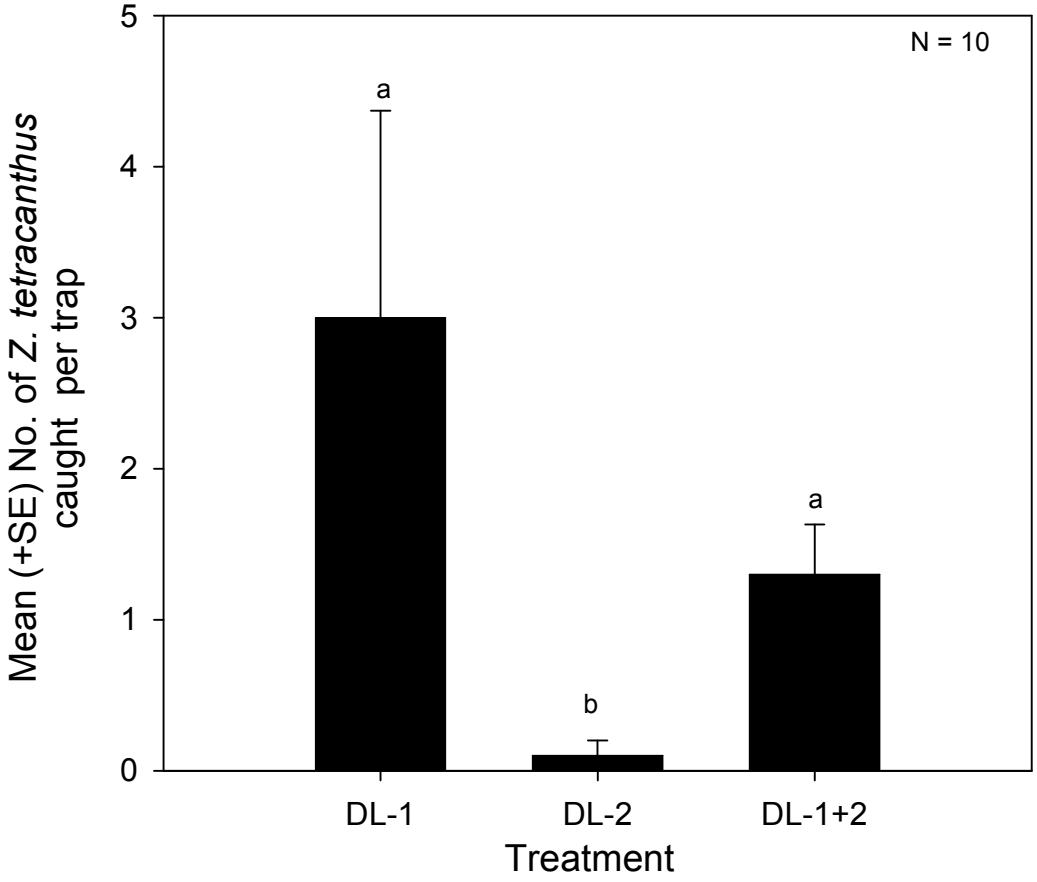

N = 10
Mean (+SE) No. of Z. tetracanthus caught per trap
0
1
2
3
4
5
a
b
a
DL-1
DL-2
DL-1+2
Treatment

Fig. 3

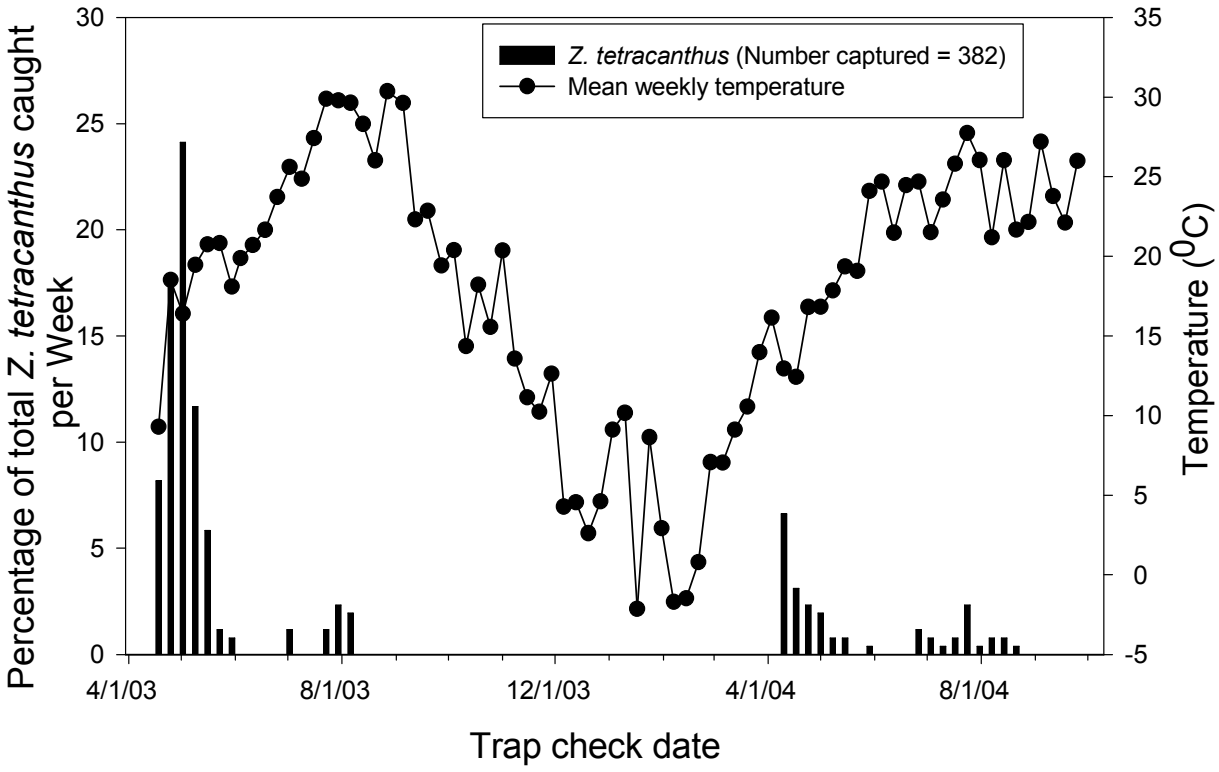

Z. tetracanthus (Number captured = 382)
Mean weekly temperature
Percentage of total Z. tetracanthus caught per Week
Temperature (0C)
Trap check date
4/1/03
8/1/03
12/1/03
4/1/04
8/1/04
0
5
10
15
20
25
30
-5
0
5
10
15
20
25
30
35

Fig. 4

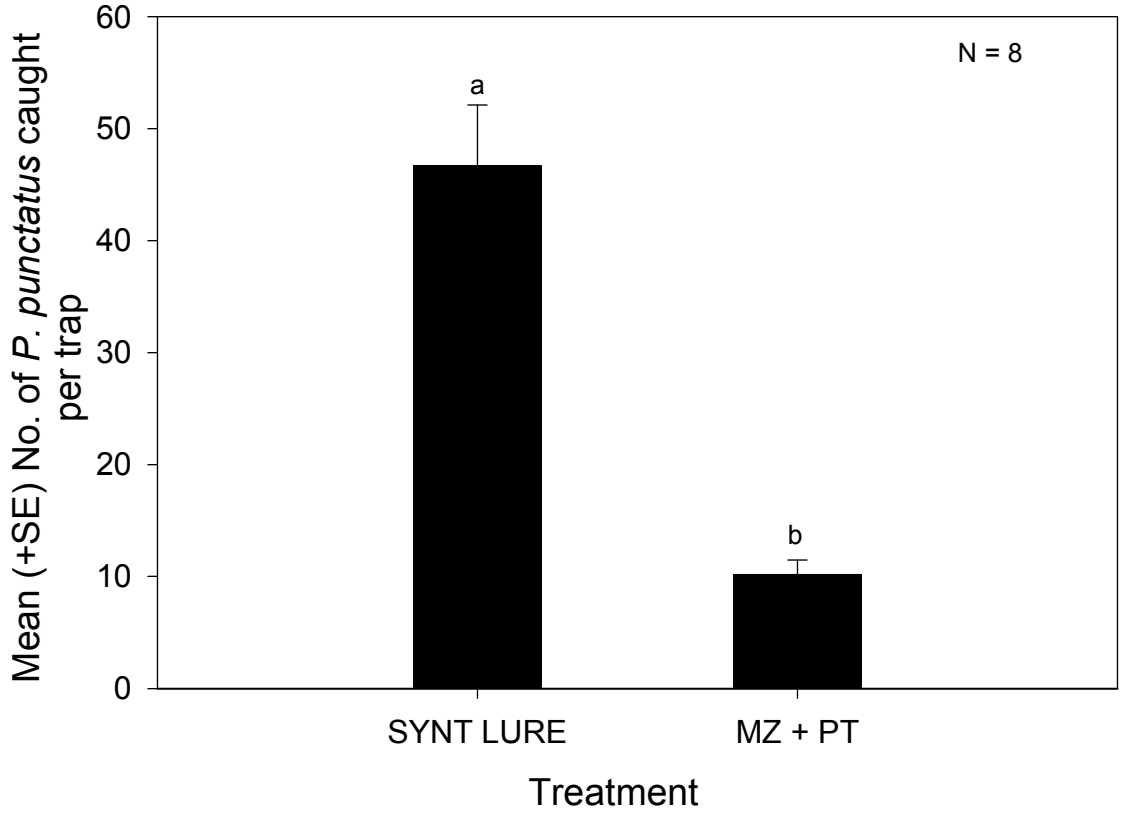

60
50
40
30
20
10
0
Mean (+SE) No. of P. punctatus caught per trap
N = 8
a
b
SYNT LURE
MZ + PT
Treatment

Fig. 5

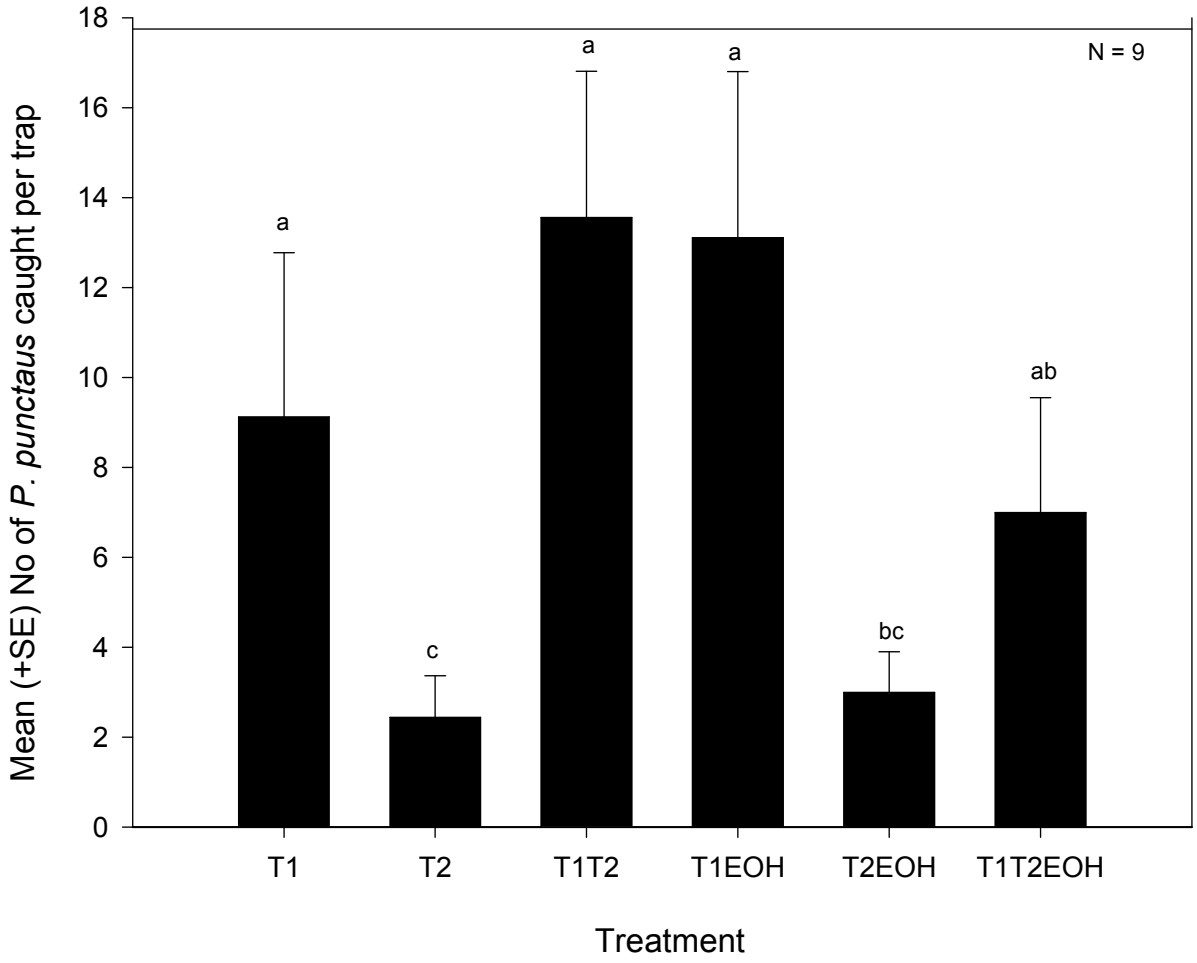
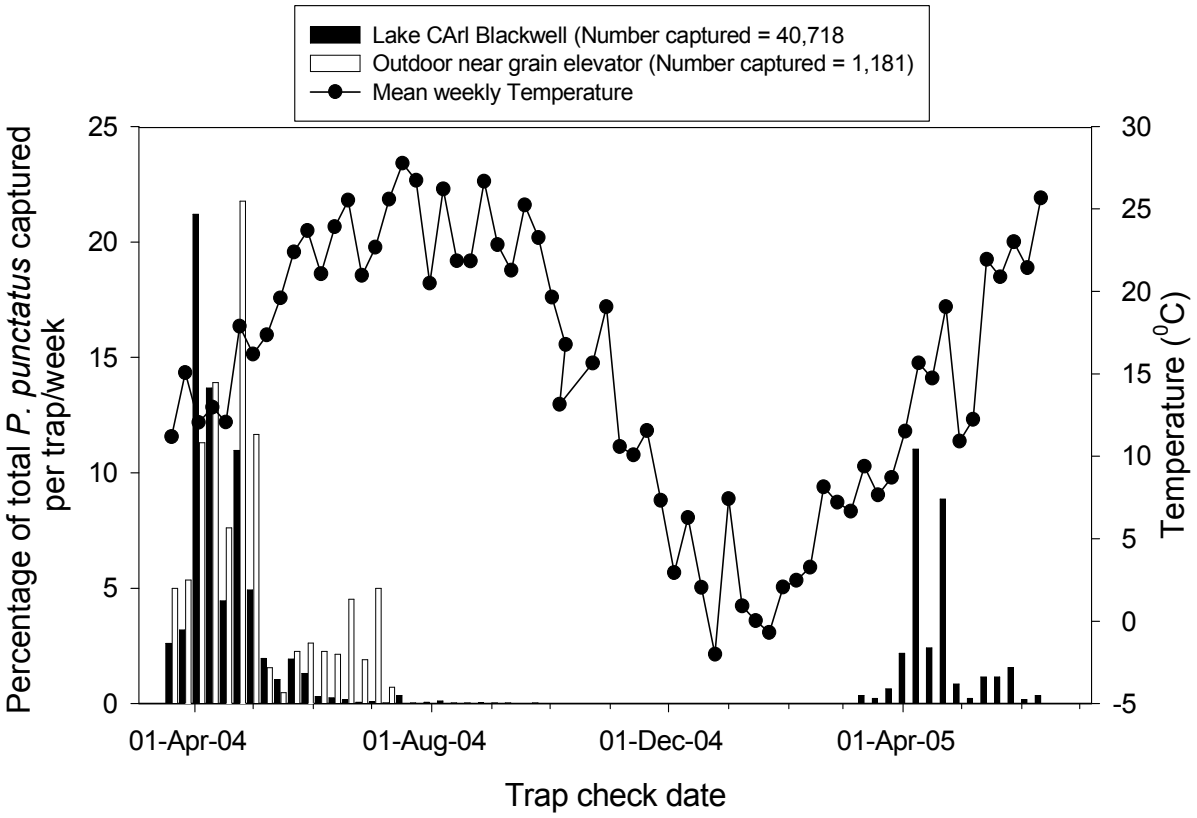

Mean (+SE) No of *P. punctaus* caught per trap
18
16
14
12
10
8
6
4
2
0
a
c
a
a
bc
ab
N = 9
T1
T2
T1T2
T1EOH
T2EOH
T1T2EOH
Treatment

Fig. 6


Lake CArl Blackwell (Number captured = 40,718
Outdoor near grain elevator (Number captured = 1,181)
Mean weekly Temperature
Percentage of total P. punctatus captured per trap/week
Temperature (0C)
0
5
10
15
20
25
30
-5
01-Apr-04
01-Aug-04
01-Dec-04
01-Apr-05
Trap check date

CHAPTER V

POTENTIAL HOST AFFINITIES FOR THE LESSER GRAIN BORER *RHYZOPERTHA DOMINICA* (F.) (COLEOPTERA: BOSTRICHIDAE): NON-GRAIN HOST EFFECTS ON REPRODUCTION AND PHEROMONE-MEDIATED HOST PLANT ORIENTATION

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Potential host affinities for the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae): non-grain host effects on reproduction and pheromone-mediated host plant orientation.

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Abstract

Behavioral responses of male and female *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to odors from pulverized wheat seeds, peanuts, cowpeas, potato tubers, acorns and twigs from cedar and pine, were compared in dual-choice, still-air bioassays. We investigated the reproductive fitness of *R. dominica* on five of the seven plant tissues (wheat, peanuts and cowpeas, dried potato tubers and acorns). A field experiment was also conducted to investigate responses of dispersing *R. dominica* to semiochemicals emitted by live males placed on different plant species. Results showed that both sexes of *R. dominica* responded to plant volatiles, but attraction was strongest to seeds of wheat, a plant species judged to be most suitable for beetle development due to the number of progeny produced on the plant species. Similarly, insects reared on wheat were heavier than those reared on less suitable materials. In general, behavioral responses by males to plant volatiles were faster than responses by females. Responses of conspecifics to aggregation pheromones produced by males feeding on different host materials were skewed toward females although both sexes were attracted. Male *R. dominica* feeding on wheat recruited more conspecifics than beetles feeding on less suitable hosts (acorns, cowpeas, peanut and potato tubers)

Keyword: Aggregation pheromone, lesser grain borer, host odor, plant species.

Introduction

THE LESSER GRAIN BORER, *Rhyzopertha dominica* F. belongs to the Coleoptera family Bostrichidae. Members of this family are wood and twig borers (Potter 1935), but *R. dominica*, has become facultatively adapted to feeding on dry starchy food, especially stored cereals (Potter 1935). Suitable food sources include grains of wheat, maize, rice and sorghum (Hagstrum et al. 1999). Feeding by adult and larvae of *R. dominica* results in "insect damaged kernels" (IDK), which cause wheat consignments to be classified as "sample grade" and can lower the value of grain when sold (Federal Grain Inspection Service 1997). Unlike other stored grain insect pests such as *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Callosobruchus maculatus* (Coleoptera: Bruchidae), which will commonly infest ripening crops in the field before harvest (Rees 2004), *R. dominica* rarely infests crops in the field prior to harvest (Edde unpublished data, Phillips unpublished data). However, *R. dominica* has been reported to infest grain, in supposedly clean stores, within weeks or months after storage (Gates 1995). Movement of this species from non-agricultural habitats to stored grain has been attributed to a strong flying ability (Winterbottom 1922) and the ability to detect volatile stimuli originating from grain stores (Dowdy et. al. 1993). In addition to the role of plant volatiles as attractants, pheromones promote aggregation of *R. dominica* on located food sources. The aggregation pheromones (S)-(+)-1-methylbutyl (*E*)-2-methyl-2-pentenoate (designated dominicalure-1 or DL-1) and (S)-(+)-1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate (designated dominicalure-2 or DL-2) are produced by male beetles (Williams et al. 1981). Both sexes respond strongly in field and laboratory settings to synthetic blends of DL-1 and DL-2.

Aggregation pheromones of *R. dominica* may act synergistically with host volatiles (Dowdy et al. 1993, Bashir et al. 2001); however, the manner in which *R. dominica* utilizes plant volatiles, with or without pheromones, during host finding is not fully understood. It is possible that *R. dominica* may not be able to discriminate between suitable and less suitable host plants solely on the basis of plant volatiles (Bashir 2000, Bashir et al. 2001), implying a costly decision in the form of adult mortality during host finding processes. However, these and other studies (e.g. Dowdy 1993, Mayhew 1994) on host finding behavior in *R. dominica* involved few plant species (< 3) and were conducted employing short-range (walking) bioassays under laboratory conditions. Because *R. dominica* is highly polyphagous (Potter 1935, Linsley 1944, Wright et al. 1990) and a strong flier, there is need for further studies on a wider range of possible host plants under long range orientation conditions that necessitate flying to suitable conditions. These studies will provide essential information on the role of plant volatiles and aggregation pheromones in host finding processes in *R. dominica*, and help in developing models on how readily the species can complete the entire host plant finding sequence in the field. An understanding of the interactions between *R. dominica* and cues employed in the host finding process may be of practical value in developing a semiochemical-based trapping system for the species.

This study had three objectives. The first was to compare the abilities of *R. dominica* to orient to plant volatiles as cues at close and long range for the purpose of selecting plant species that are suitable hosts. The second objective was to determine the suitability of certain host plant material for reproduction. The third objective was to

investigate responses of conspecifics to natural pheromone released by male *R. dominica* feeding on different food sources in the field.

Materials and Methods

Insect culture. To ensure that adults of *R. dominica* used in these experiments had not been exposed to volatiles arising from plant tissues to be tested in bioassays, a colony was started from field-collected insects caught in pheromone-baited traps near a grain storage facility for two generations. The insects were maintained on yellow maize (*Zea mais* L.) seeds at 29-30°C and 65% relative humidity and on 12-hour light and 12-hour dark cycle. For easy removal of beetles of known age and sex, *R. dominica* were reared for one generation in a subculture of mixed maize flour and brewer's yeast (95:5). Parent insects were allowed to lay eggs then removed after one week. Following larval development, pupae were sifted from the culture medium (three weeks after removal of parent adults) and placed in separate vials until adult emergence. The sex of adult *R. dominica* was determined as they emerged by gently squeezing the abdomen to cause extrusion of their genitalia, which were viewed under a dissecting microscope (Crombie 1941).

Plant materials. Tissues of seven plant species were used in the experiments including wheat seeds [*Triticum aestivum* L. (Poaceae)]; peanut seed [*Arachis hypogea* L. (Leguminosae)] and cowpea seeds [*Vigna unguiculata* (L.) Walp. (Papilionaceae)]; potato tubers [*Solanum tuberosum* L. (Solanaceae)]; post oak seeds [*Quercus stellata* Wangenh (Fagaceae)]; woody tissues from 1-2 year old terminal branches of Eastern Red cedar [*Juniperus virginiana* (Cupressaceae)] and Loblolly pine [*Pinus taeda* L.

(Pinaceae)]. These plant species have been reported as possible hosts of *R. dominica* (Potter 1935, Wright et al. 1990). Freshly harvested seeds of hard red winter wheat were obtained from farms located near Stillwater, OK. Unprocessed shelled peanut and cowpea seeds were obtained from a commercial grocery store. Acorns, cedar and pine twigs were harvested from live trees at a local field site (36°03′N; 097°10′W), which is a naturally self-regenerating forest. Due to size and the high water content of acorns, potato tubers and twigs, the plant materials were cut into small pieces and dried. To obtain dried tissues of acorn and potato, the plant materials were cut into cubes (≈7 mm^3) and sun-dried. The twigs were cut into pieces (≈1cm long), spread thinly on a tray and dried outdoors under natural sunlight.

Experiment 1. Dual-choice, still-air bioassay. Volatiles from wheat, peanuts, cowpeas, potato tubers, acorn, and twigs from cedar and pine, were used to compare the responses of male and female *R. dominica*. To ensure plant tissues used as a source of volatiles were of similar size, plant materials were pulverized using a mechanical grinder, and sifted using a # 21 sieve (Seedburo Equipment Co., Chicago, IL.). Evaluations were performed in a room maintained at 28-29°C, and a relative humidity of about 65%, and under dim light supplied with a 60-watt red iridescent bulb. Moisture content of plant materials used in this and subsequent experiments in the study ranged from 12.6-14.1% at the start of the experiment. Moisture equilibration was achieved by placing Petri dishes containing pellets in humidifiers containing a saturated sodium chloride solution.

The bioassay arena adopted was similar to the one used by Prokopy et al. (1995). A glass Petri dish (9 cm inner diameter, 1.5 cm deep) was modified with two holes in the floor, about 0.6 cm diameter that were spaced 5 cm apart through the middle of the dish,

about 2.5 cm from the center. A glass vial (1 cm dia. x 4.5 cm) was centered under the opening of each hole. About 1.5 g of the materials to be tested was weighed into one of the vials; the second vial was left empty (control). The holes were covered with wire mesh screens (16 openings/cm) to prevent beetles from falling into the vial. The top of the wire screen was lined with filter paper to facilitate beetle movement. Two circular cuts were made in the filter paper to allow access to the holes in the Petri dish.

The insects used in the study were starved for 24 hour before use in the experiment. A single insect was introduced into the middle of the arena through an inverted glass funnel (2.5 cm dia.) placed in the center of the Petri dish. The assay commenced when the funnel was removed. Forty beetles (20 males and 20 females) were tested individually per treatment. In a preliminary test, *R. dominica* released in the center of the bioassay arena move toward the source of plant odor gradually, and became settled in a mean time of about 13 minutes, thus fifteen minutes was adopted as the maximum time allowed for a beetle to respond to an odor source. A positive response was scored when a beetle walked to the edge of a hole and remained there for more than 10 seconds. Beetles that went straight to the stimuli source zone or unbaited control immediately after release were not included in data analysis. Filter paper lining the arena was replaced after every replication. Petri dishes and wire mesh screens were cleaned with acetone and dried after each test. The time taken by individual *R. dominica* to arrive at an odor source was used as the criterion to measure preference for particular volatiles. Data (time required for positive responses) in the experiment were analyzed as a two-factor experiment in which the main factors were plant species and beetle sex.

Experiment 2. Reproduction on tissues of different plant species.

Attempts to rear *R. dominica* on all seven plant tissues used in Experiment 1 proved difficult on loose pulverized materials and on pellets made from woody tissues of cedar and pine. As a result, reproductive rate of *R. dominica* was compared on only five of the plant species: seeds of wheat, peanuts cowpeas, and dried cubes of potato and acorns. Twenty adults (16 females and 4 males) a week old were released in 100 ml jars containing 50 g of the plant materials in four replicates. To facilitate feeding and egg depositions, for each plant species about 5 g of the plant materials was milled and added to the jar. The lids of the jars were perforated and covered with wire mesh to prevent escape of beetles and provide aeration. The jars were arranged in a randomized complete block design in which the jars were arranged in four blocks. The insect growth chamber was maintained at 30°C, and a relative humidity of 65%. Preliminary results indicate that the shortest time to adult F_1 emergence was on wheat, occurring in 28 days after initial release. The longest development time was 34 days, occurring in potato and cowpea. Thus, the jars were left undisturbed until 35 days when they were sifted to remove all parent adults and adults of the F_1 generation that were present at that time. The plant materials and frass produced were replaced in the jars, returned to the growth chamber, and reexamined another 35 days later for remaining F_1 adults and F_2 generation adults. Thereafter, beetles were removed every four days until no further progeny emerged from any treatment**.** The difference between the sum of emerged F_1 and F_2 adults and number of parent adults used as sources of infestation was calculated as the 'number of beetles emerged' for a given jar. Fresh body weight of emerged male and female beetles from

different treatments was determined to the nearest 0.001 mg, using a Sartorius electronic microbalance type M3P (Satorius Instruments, McGaw Park, IL).

Data on progeny production on different plant tissues was analyzed as a one-factor experiment, and those for weight of beetles emerged as a two-factor experiment in which the main factors were plant species and beetle sex.

Experiment 3. Responses by *R. dominica* to aggregation pheromones. A field experiment was conducted to investigate response of conspecific male and female *R. dominica* to host odor and natural pheromones, if any, emitted by males as they fed on different plant species. The study was conducted from 11 July to 15 August 2003 at Lake Carl Blackwell in central Oklahoma (36°07′N; 097°13′W). Details on characteristics of the study site have been described in a previous study (Edde et al. 2005).

To generate aggregation pheromones, male beetles (less than a week old) were placed singly on 10 g of coarsely ground wheat, cowpea and peanut seeds, dried cubes of potato and acorns measured into a cylindrical copper mesh screen cage (10 cm long and 1 cm dia) that was plugged at both ends with rubber stoppers. The insects were conditioned on the test materials for 72 hours before being used in the study. The copper mesh screen was adequate to retain beetles and plant materials, and easily allowed the escape of volatile chemicals. Insect cages were attached inside the middle of Lindgren four-unit funnel traps (Lindgren 1983).The traps were hung from vertical polyvinyl chloride pipe stands that were inserted into the soil, about 1.7 m above the ground, and spaced 15- 20 m apart. Another set of traps were baited with food cages containing plant materials only, with no beetles. Control traps were left blank i.e. without infested plant materials or food cages. Soapy water was used in the collection cups to prevent captured insects from

escaping. The experiment was repeated every week during which food cages and beetles were replaced, and captured insects were removed and counted. The study lasted for five weeks. Sexes of captured beetles were determined in order to investigate if pheromones released by beetles feeding on different food sources affected sex ratio of responding *R. dominica*. Generally, 45% or more of the insects captured in each treatment were sexed, but this number differed based on insect condition.

The study was conducted as a randomized complete block design in which each week represented a block. Treatments (host plants with males, host plants alone, and blank traps) were replicated once and randomly arranged within rows of traps in a week. Data on the numbers of beetles captured were analyzed as a two-factor experiment in which the main factors were plant species and beetle sex.

Statistical Analysis. Data were analyzed using SAS PROC MIXED (SAS Institute, 2001). Blocks (Experiment 1 and 2) and weeks (Experiment 3) were considered as random effects in the respective mixed models and therefore included in the RANDOM statement within the PROC MIXED code. Before data analysis, count data were transformed using the Log (X +1), in order to satisfy the assumptions of normality and homogeneity of variance (Zar 1999). Actual means and standard errors are presented in the text, tables, and figures. Tukey's Studentized range test was used to separate means (Tukey 1953).

Results

Responses of *R. dominica* to volatiles from different plant species. Male and female *R. dominica* responded positively to plant volatiles. Of the 342 *R. dominica* (male and females) assayed, 82% were attracted to plant volatiles in our bioassay. Six percent of the

beetles move toward the blank control, but none of these stayed longer than 7 seconds. The remaining 12% comprised beetles that went straight to the stimuli source zone or unbaited control immediately after release, and these were not included in the analysis, and those that did not respond to either plant volatiles or control during the 15 min allotted for each trial.

A two-factor analysis of variance indicated significant ($F = 2.73$; df = 6, 266; $P < 0.05$) interactions between plant species and sex of *R. dominica* for the amount of time required to locate sources of volatiles. Males reacted faster than females to locate sources of volatiles from wheat (t-test = 4.57; df = 266; $P < 0.001$), cowpea (t-test = 2.52; df = 266; $P < 0.05$) and peanut (t-test = 2.72; df = 266; $P < 0.01$). However, responses to volatiles from acorn, pine, cedar and potato were not significantly different for the sexes (Fig. 1).

Similarly, the main effect of sex was significant ($F = 20.18$; df = 1, 266; $P < 0.001$) for the entire experiment, such that males of *R. dominica* exhibited a more rapid behavioral response to plant volatiles than females. The main effect of plant species on responses of *R. dominica* was significant ($F = 8.33$; df = 6, 266; $P < 0.001$). Response by *R. dominica* was most rapid to volatiles from wheat and cedar (Fig. 1). Volatiles from acorn elicited a slower response, which was not significantly different from responses observed to cowpea, peanut and pine.

Reproduction on tissues of different plant species. *Rhyzopertha dominica* chewed holes in peanut seeds, and two beetles (a male and a female) were still alive after two months, however, no reproduction occurred at all on peanut. Since this treatment had no variability in responses by the insects, it was not included in the data analysis because of

possible interference with the assumption of homogeneity of variances in the model (Reeve and Strom 2004). For the other plant species, reproduction of *R. dominica* varied significantly ($F = 314.88$; df = 3, 12; $P < 0.001$). Significantly higher reproduction was observed on wheat, than on acorn, cowpea and potato (Table 1). Reproduction was not significantly different on acorn, cowpea and potato (Table 1). However, when reproduction on wheat was excluded from the analysis, reproductive rates were significantly ($F = 9.42$; df = 2, 9; $P < 0.01$) different among the three remaining plant species, acorn, cowpea and potato. Application of post hoc test for mean number of beetles emerged revealed that reproduction rate on acorn and cowpea were statistically similar, and that each of these plant species resulted in significantly greater progeny production than potato.

A two-factor analysis of variance showed no significant ($F = 0.55$; df = 3, 139; $P < 0.65$) interaction between plant species and sex in the weight of emerged adults. Similarly, there were no significant differences between the mean weights of emerged male or female *R. dominica* within plant species (Table 1). However, the main effect of plant species was significant ($F = 0.55$; df = 3, 140; $P < 0.001$). Mean weights of beetles raised on wheat were heavier than those reared on other plant species (Table 1). Weight of emerged *R. dominica* on acorn, cowpea and potato were similar. Based on results presented in Table 1, plant species may be grouped as suitable (wheat), moderate (acorn, cowpea and potato) and unsuitable (peanut) for *R. dominica* reproduction.

Recruitment potential of male *R. dominica* feeding on different plant tissues. Unbaited traps or those baited with plant materials alone did not capture any *R. dominica*, and were not included in the initial data analysis. In contrast, conspecific male and female

R. dominica were attracted to traps baited with live males feeding on plant materials. No significant ($F = 1.00$; df = 4, 36; $P = 0.42$) interaction between plant species and sex of beetles captured in traps baited with pheromones was detected.

Traps baited with live male *R. dominica* on wheat captured the highest number of beetles (Fig. 2). Trap captures with male beetles on acorns were similar to those on cowpea or potato. Traps with live *R. dominica* feeding on peanut caught the lowest numbers (Fig. 2). When the number of beetles caught in traps with host plants plus males were compared to the zero counts for response on host plant only, the responses were significant for wheat (t-test = 4.99; df = 4; $P < 0.01$), acorns (t-test = 3.21; df = 4; $P < 0.05$), cowpeas (t-test = 5.88; df = 4; $P < 0.01$), and potato (t-test = 4.81; df = 4; $P < 0.01$). These results suggest that male *R. dominica* feeding on plant tissues produce pheromones that recruited conspecifics to the traps.

ANOVA showed that the main effect of sex of beetles captured in traps baited with pheromones (i.e. live males feeding on plant materials) was significant ($F = 10.41$; df = 1, 36; $P < 0.001$). Pooled data of responses across plant species showed that females exhibited stronger responses than males to trap baited with natural pheromones ($t = 3.23$; df = 36; $P < 0.01$). Within plant species, however, responses by female and male beetles were not significantly different for traps baited with live beetles on wheat (t-test = 1.63; df = 4; $P = 0.14$) acorn (t-test = 1.96; df = 4; $P = 0.09$), cowpea (t-test = 0.75; df = 4; $P = 0.48$); or peanut (t-test = 0.00; df = 4; $P = 1.00$) except on potato where the number of females captured was significantly (t-test = 2.04; df = 4; $P = 0.02$) higher than the number of males caught.

Discussion

Both sexes of *R. dominica* responded positively to volatiles emitted from the plant species tested. We cannot rule out the possibility that *R. dominica* might respond to water in the plant tissues vs. the dry blank control; however, variation in response to plant odor (Fig. 1) suggests there is more of an odor effect than water only. Response by *R. dominica* was most rapid to volatiles from wheat, a plant species judged to be the most suitable host because of greater reproduction and heavier weights of emerged beetles relative to other plant species tested (Table 1). Although, *R. dominica* was able to feed on peanut, as evidenced by feeding holes and frass produced, no progeny were produced. Our findings suggest that *R. dominica* may be capable of using olfactory cues to discriminate between suitable and unsuitable plant species during the host-finding process.

Our observations are similar to those of Scholz et al. (1997) who worked with *Prostephanus truncatus*, another Bostrichidae and a primary pest of stored maize (*Zea mais* L.) and cassava (*Manihot esculenta* Crantz). Using a bioassay method similar to that of Bashir (2000), Scholz et al. (1997) observed that *P. truncatus* were attracted to volatiles from maize and cassava (*Manihot esculenta* Crantz), but showed no response to volatiles from cowpea, a non-host plant. Since a high cost in the form of mortality to adults or wasted oviposition attempts may result from choosing the wrong host plant, it is possible that species feeding on stored grains have evolved elaborate mechanisms directed toward efficient utilization of olfactory cues to select suitable host plants. The phenomena of using host associated volatiles to discriminate between competing host

odors has been demonstrated among wood boring beetles in the family Scolytidae (Byers 1995, Pureswaran and Borden 2005).

Results from Experiment 1 also showed that attraction of *R. dominica* to stimuli from cedar (gymnosperm) was not significantly different from observations on wheat. This observation may not be surprising. The family Bostrichidae, to which *R. dominica* belongs, consists primarily of stem and twig boring beetles and this species has been reared successfully on seeds of several tree species (Potter 1935, Wright et al. 1990), indicating that present day feeding on cereals and other starchy food may be a secondary adaptation (Potter 1935). Although, *R. dominica* may have become wholly adapted to a cereal diet, the insect may have retained or evolved genetic capability to use olfactory cues to discriminate between competing odors among its natural hosts. The current findings parallel those by Bashir (2000) who observed that *R. dominica* showed no significant preference among volatiles from peanut, wheat and maize. In agreement with Bashir (2000) suggestions, *R. dominica,* presumably, may have perceived cedar volatiles attractive because the tree species could have been one of its original hosts or perhaps cedar volatiles consist of chemical components identical to those found in its original or adopted hosts. For example, findings by Hougen et al. (1971) suggested that different plant species may emit similar volatiles, but in different quantities. If cedar was among *R. dominica* original host plants, then it is likely the insect might have lost ability to adapt to changes in cedar chemistry following adaptation to stored grains (Linsley 1944, Byers 1995), hence the inability to reproduce on pulverized tissues or pellets made from this tree species.

In contrast to results from Experiment 1, not a single *R. dominica* was captured in traps where plant materials (wheat, potato, acorn and peanut) alone were used as attractants in Experiment 3. However, beetles were captured in traps baited with infested plant materials. The lack of consistency in results of the two experiments may be a consequence of the limited amount of volatiles emitted from baits used in traps. Volatiles emitted may have been insufficient enough to elicit a response in their natural environment, whereas the concentration of volatiles was sufficient in the enclosed Petri dish walking bioassay. In previous field investigations on odor-based host finding behavior of *R. dominica*, it was shown that volatiles were emitted from bulk storage of wheat (Barrer 1983). It is likely, therefore, that the failure to capture *R. dominica*, even on wheat, in our field trapping might be due to the low quantity (10 g) of grain used as an attractant. A more likely explanations however, is that attraction to plant odors observed in the walking bioassay probably suggests a short-range effect in the role of primary attraction in host finding behavior of *R. dominica*. Perhaps, only a small proportion of dispersing *R. dominica*, if any, would need to employ primary attraction for host finding, because conspecifics can exhibit strong orientation to aggregation pheromones released by male beetles. The male produced aggregation pheromones, therefore, may be considered the most important stimuli for host plant location in *R. dominica*, and may play a crucial role in subsequent aggregation by conspecific male and female *R. dominica*. The phenomenon of secondary attraction, i.e. orientation to aggregation pheromones, has been suggested for other stored product bostrichid pests *P. truncatus* and *Dinoderus bifoveolatus* (Scholz et al. 1997, Fadamiro et al. 1998, Borgemeister et al. 1999), and is very common among bark beetle species (e.g. Byers 1995, Pureswaran and

Borden 2005). Most of the above-mentioned species have strong aggregation pheromones, and many exhibit weak or lack of attraction to host plant volatiles under field conditions (Scholz et al. 1997, Fadamiro et al. 1998, Borgemeister et al. 1999, Byers 1995, Pureswaran and Borden 2005).

Attraction of male and female *R. dominica* to aggregation pheromones was highest when males were placed on wheat, but only moderate for when males were placed on acorn, potato and cowpea, and low for males placed on peanut. It is not surprising that this ranking parallels the observed results on reproductive rate of *R. dominica* on the plant species. These observations support the view that male signalers may have evolved behavioral adaptations to vary quantity or ratio of chemical signals to indicate to conspecifics the suitability of located resources for feeding and reproduction (Mayhew 1994, Bashir 2000, Bashir et al. 2003). Very little is known on the mechanism of how host plant chemistry would affect attractiveness of pheromone signals in *R. dominica*. In a laboratory study, Bashir et al. (2003) showed that the absolute quantities of the aggregation pheromones (DL-1 or DL-2) produced were lowered when male *R. dominica* were moved from suitable host to unsuitable host materials, but that pheromone release rate and ratio were restored when the move was reversed. Apparently, males respond by emitting less pheromone on unsuitable host, making it less likely that migrant conspecifics will detect the pheromones (Baker and Kuenen 1982, Roelofs 1978, Byers 1995). The phenomena of host plant effect on the attractiveness of the signaler is also known to occur in *Ips typographus* (Coleoptera: Scolytidae) (Birgersson 1989). We are currently investigating pheromone release rate on the plant species used in Experiment 1 in order to provide additional evidence for

results obtained in our field study. In addition to modifying quantity of pheromone emission, male signalers may vary the quality of the chemical signals released. Recently, Seitz and Ram (2004) showed that many volatile compounds in addition to DL-1 and DL-2 are produced by *R. dominica,* and it is likely, therefore, that regulation of the release of one or more of these compounds in response to host plant quality may indicate to conspecifics the suitability of located resources for feeding and reproduction.

The fact that both male and females responded to pheromones emitted by males feeding on different plant species confirmed that *R. dominica* releases an aggregation pheromone (Williams et al. 1981). However, analysis of data for sexes across plant species showed that dispersing female *R. dominica* were more strongly attracted than males to pheromones. This is similar to previous observations on the species (Edde et al. 2005), and on some stored-product and wood boring beetles that utilize male-produced aggregation pheromones (Plarre and Vanderwel 1999, Cronin et al. 2000, de Groot and Nott 2001). The level of response by beetles in the field to the pheromone produced by just one male *R. dominica* in each trap in this experiment is interesting because the quantity of pheromone produced is many orders of magnitude less than that emitted by synthetic pheromone lures (Edde unpublished). We believe that this is the first report of response of wild *R. dominica* to naturally produced pheromones.

Pheromone production by male *R. dominica* in the absence of females, and the observation that males respond more strongly than females to plant odors, supports the suggestion that males may be more sensitive to host stimuli than females and/or male *R. dominica* may be the sex that locates suitable hosts where they attract females as potential mates (Bashir 2001), but other males exploit the signal for locating assembled

females and resources (Phillips 1997, Landolt 1997, Landolt and Phillips 1997). In this sense, the role of aggregation pheromones of *R. dominica* may include mate and resource finding.

The data presented here demonstrate that both sexes of *R. dominica* could respond to plant volatiles, but attraction parallels the relative suitability of the plant species to support feeding and reproductive fitness of the insect. In general, behavioral responses by males to plant volatiles were faster than responses by females; however, responses of conspecifics to pheromones were skewed toward females. Male *R. dominica* feeding on wheat recruited more conspecifics than beetles feeding on less suitable hosts: acorn, cowpea, peanut and potato. The observation that *R. dominica* was able to reproduce and also produce aggregation pheromones on non-cereal crops such as cowpeas and dried potato tubers and acorns is an indication that these plant species could act as alternate hosts for the pest in the absence of preferred hosts like wheat and/or maize.

Acknowledgement

The authors are grateful to Drs. Richard Berberet and Tom Royer for reviewing an early draft of the manuscript. The USDA CSREES Risk Avoidance and Mitigation Program, under Agreement No 2000-05385, funded this project.

References

Barrer, P. M. 1983. A field demonstration of odour-based, host-food finding behaviour in several species of stored grain insects. J. Stored Res. 19: 105-110.

Baker, T. C., and L. P. S. Kuenen. 1982. Pheromone source location by flying moth: a supplementary non-anemotactic mechanism. Science. 216: 424-427.

Bashir, T. 2000. Pheromone communication and host finding behavior of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) PhD thesis, University of Greenwich, U.K.

Bashir, T., L. A. Birkinshaw, D. R. Hall, and R. J. Hodges. 2001. Host odours enhance the responses of adult *Rhyzopertha dominica* to male-produced aggregation pheromone. Entomol. Exp. Appl. 101: 273-280.

Bashir, T., R. J. Hodges, L. A. Birkinshaw, D. R. Hall, and D. I. Farman. 2003. Phenotypic plasticity of *Rhyzopertha dominica* pheromone signaling: the effects of different hosts and presence of Conspecific females on male produced aggregation pheromone. J. Chem. Ecol. 29: 945-959.

Birgersson, G. 1989. Host tree resistance influencing pheromone production in *Ips typographus* (Coleoptera: Scolytidae). Holarctic. Ecol. 451-456.

Borgemeister, C., K. Schaefer, G. Goergen, S. Awande, M. Setamou, and H. -M. Poehling. 1999. Host-finding behavior of *Dinoderus bifoveolatus* (Coleoptera: Bostrichidae), an important pest of stored cassava: The role of plant volatiles and odors of conspecifics. Ann. Entomol. Soc. Am. 92: 766-771.

Byers, J.A. 1995. Host tree chemistry affecting colonization in bark beetles. *In* R. T. Cardae, and W. J. Bell (eds.), Chemical Ecology of Insects 2. Chapman and Hall. New York.

Crombie, A. C. 1941. On oviposition, olfactory conditioning and host selection in *Rhyzopertha dominica* Fab. (Insecta, Coleoptera). J. Exp. Bio. 18: 62-79.

Cronin, J. T., J. L. Hayes, and P. Turchin. 2000. Evaluations of traps to monitor southern pine beetle aerial populations and sex ratios. Agric. For. Entomol. 1: 69-76.

De Groot, P., and R. Nott. 2001. Evaluation of six trap designs to capture pine sawyer beetles (Coleoptera: Cerambycidae). Agric. For. Entomol. 3: 107-111.

Dowdy, A. K., R. W Howard, L. M. Seitz, and W. E. McGaughey. 1993. Responses of *R. dominica* (Coleoptera: Bostrichidae) to its aggregation pheromone and wheat volatiles. Environ. Entomol. 22: 965-970.

Edde, P. A., T. W. Phillips, and M. D. Toews. 2005. Responses of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromones as influenced by trap design, trap height and habitat. Environ. Entomol. (in press).

Fadamiro, H. Y., I. Gudrups, and R. I. Hodges. 1998. Upwind flight of *Prostephanus truncatus* is mediated by aggregation pheromone but not food volatiles. J. Stored Prod. Res. 34: 151-158.

Federal Grain Inspection Service (FGIS). 1997. Wheat. In Grain inspection handbook, Book II, grain grading procedure, USDA-GIPSA-FIGS, Washington, DC.

Gates, M.W. 1995. Population dynamics of lesser grain borer, rusty grain beetle, and *Cephalonomia waterstoni* in commercial elevators. M.S. thesis, Oklahoma State University Stillwater, Oklahoma.

Hagstrum, D. W., C. Reed, and P. Kenkel. 1999. Management of stored wheat insect pests in the USA. Integrated Pest Man. Rev. 4: 127-142.

Hougen, F. W., M. A. Quilian, and W. A. Curran. 1971. Headspace vapor from cereal grains. J. Agric. Food Chem. 19: 182-183.

Landolt, P. J. 1997. Sex attractant and aggregation pheromones of male phytophagous insects. Am. Entomol. 43: 12-22.

Landolt, P. J., and T. W. Phillips. 1997. Host plant influence on sex pheromone of phytophagous insects**.** Ann. Rev. Entomol. 42: 371-391.

Lindgren, B. S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). Can. Entomol. 115: 299-302.

Linsley, E. G. 1944. Natural sources, habitat, and reservoir of insects associated with stored products. Hilgardia 16: 187-224.

Mayhew, T. J. 1994. Pheromone production and behavioral response to pheromones in the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). MS thesis, University of Wisconsin-Madison, USA.

Phillips, T. W. 1997. Semiochemicals of stored product insects: current status and future perspective. J. Stored Prod. Res. 33: 17-30.

Plarre, R., and C. Vanderwel. 1999. Stored-product beetles, pp 149-198. *In* J. Hardie, and A. K. Minks (eds.), Pheromones of non-lepidopteran insects associated with agricultural plants. CABI, UK.

Potter, C. 1935. The biology and distribution of *Rhyzopertha dominica* (Fab.). Trans. Royal Entomol. Soc. 83: 449-482.

Prokopy, R. J., S. S. Cooley, and P. L. Phelan. 1995. Bioassay approaches to assessing behavioral responses of plum curculio adults (Coleoptera: Curculionidae) to host fruit odor. J. Chem. Ecol. 21: 1073-1084.

Pureswaran, D. S., and J. H. Borden. 2005. Primary attraction and kairomonal host discrimination in three species of *Dendroctonus* (Coleoptera: Scolytidae). Agric. For. Entomol. 16: 765-782.

Rees, D. 2004. Insects of stored products. CSIRO Publishing. Collingwood, Victoria, Australia.

Reeve, J. D., and B. L. Strom. 2004. Statistical problems encountered in trapping studies of scolytids and associated insects. J. Chem. Ecol. 30: 1575-1590.

Roelofs, W. L. 1978. Threshold hypothesis for pheromone perception. J. Chem. Ecol. 4: 685-699.

SAS Institute. 2001. SAS/STAT User's Guide, version 8, SAS Institute Inc., Cary, NC.

Scholz, D. 1997. Dispersal and host finding behavior of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). PhD dissertation. Hanover University, Germany.

Scholz, D., C. Borgemeister, W. G. Meikle, R. H. Markham, and H. M. Poehling. 1997. Infestation of maize by *Prostephanus truncatus* initiated by male-produced pheromone. Ent. Exp. Appli. 83: 53-61.

Seitz, L. M., and M. S. Ram. 2004. Metabolites of lesser grain borer in grains. J. Agric. Food Chem. 53: 898-908.

Tukey, J.W. 1953. The problem of multiple comparisons. Department of Statistics, Princeton University. Princeton, NJ.

Williams, H. J., R. M. Silverstein, W. E. Burkholder, and A. Khorramshahi. 1981. Dominicalure 1 and 2: components of aggregation pheromone from male lesser grain borer *Rhyzopertha dominica* (F). J. Chem. Ecol. 7: 759–780.

Winterbottom, D. C. 1922. Weevil in wheat and storage of grain in bags: a period of Australian experience during the war period (1915 - 1919). Government Printer, Adelaide, Australia.

Wright, V. F., E. E. Fleming, and D. Post. **1990**. Survival of *Rhyzopertha dominica* (Coleoptera, Bostrichidae) on fruits and seeds collected from woodrat nests in Kansas. J. Kansas Entomol. Soc. 63: 344-347.

Zar, J. H. 1999. Biostatistical analysis. Prentice-Hall Inc., Englewood Cliffs, NJ.

Table 1. Reproductive fitness of *Rhyzopertha dominica* (F.) reared on different plant species[1, 2]

Plant species	Mean number of emerged adults	Mean weight of emerged adult		
		Male (mg)	Female (mg)	Mean (mg)
Acorn	45.50±7.96 B	0.97±0.03 (26)	1.01±0.04 (22)	0.99±0.04 (48) B
Cowpea	32.51±1.19 B	0.97±0.04 (20)	0.96±0.04 (18)	0.96±0.03 (38) B
Potato	15.00±3.81 B	1.03±0.04 (16)	1.09±0.05 (10)	1.05±0.03 (26) B
Wheat	1043.25±56.33 A	1.31±0.02 (18)	1.30±0.02 (32)	1.30±0.02 (50) A

[1] Means ± SE within columns followed by same letter are not significantly different at $P < 0.05$
[2] Number in parenthesis represents number of observations.

Figure legends

Figure legends

Fig. 1. Mean (± SE) time taken by *R. dominica* to locate stimuli from different plant tissues in a dual-choice, still-air bioassay. Data represent observations by both sexes combined. Bars with the same letter above them are not significantly different ($\alpha = 0.05$). Bars with asterisk above them indicate that mean time required for males to locate stimuli was significantly less than that required by females (***$\alpha < 0.001$, **$\alpha < 0.01$, *$\alpha < 0.05$).

Fig. 2. Responses by *R. dominica* to natural pheromones in Experiment 3. Bars with the same letter above them are not significantly different ($\alpha = 0.05$). Bar with asterisk above it indicate that mean number of females captured was significantly higher than the number of males caught (*$\alpha < 0.05$).

Fig. 1

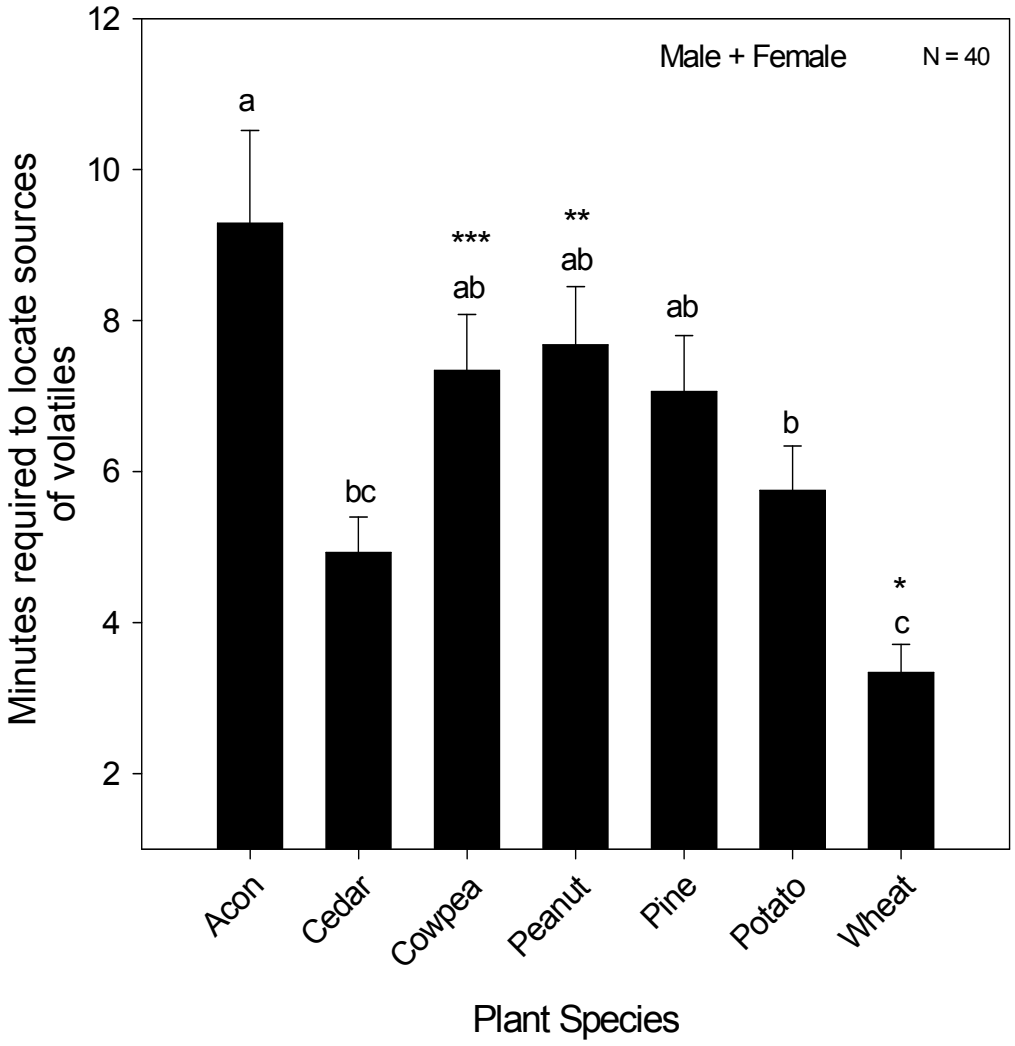
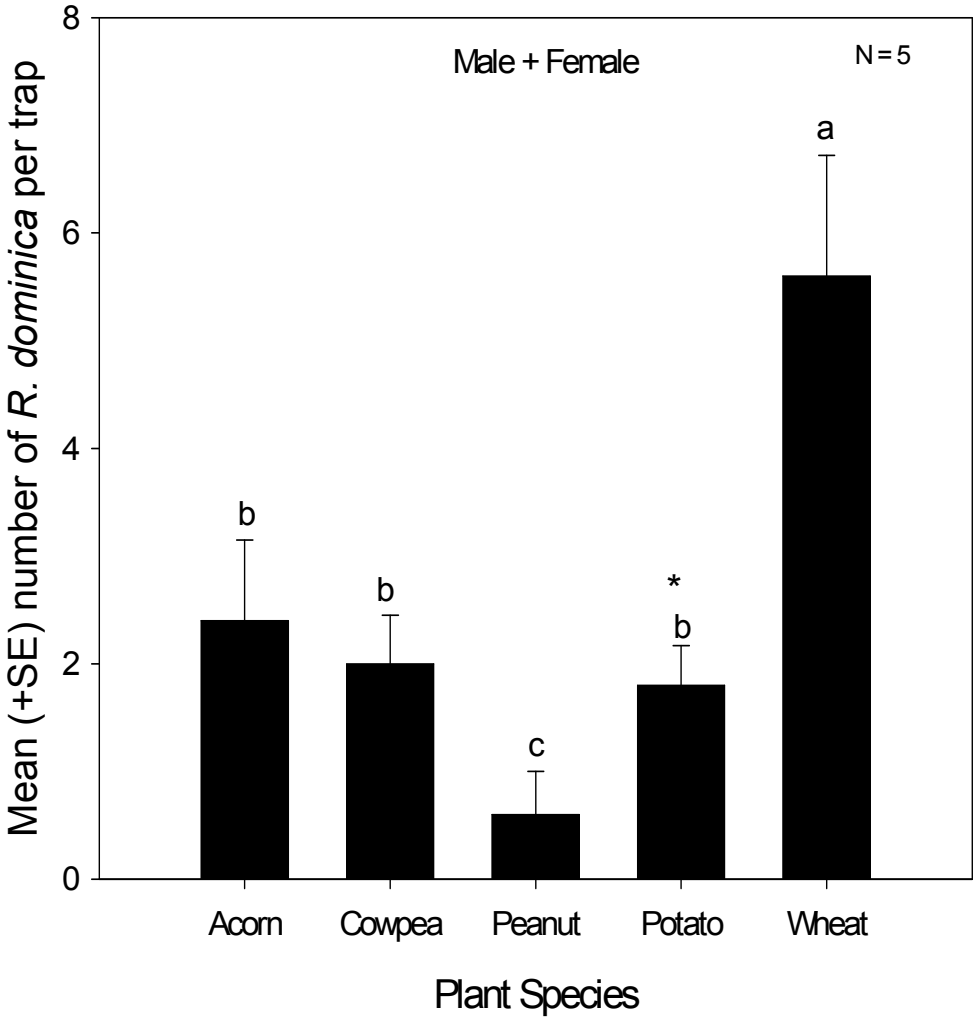

Male + Female
N = 40
Minutes required to locate sources of volatiles
12
10
8
6
4
2
a
bc
***
ab
**
ab
ab
b
*
c
Acon
Cedar
Cowpea
Peanut
Pine
Potato
Wheat
Plant Species

Fig. 2


Male + Female
N = 5
Mean (+SE) number of R. dominica per trap
0
2
4
6
8
b
b
c
*
b
a
Acorn
Cowpea
Peanut
Potato
Wheat
Plant Species

CHAPTER VI

PHEROMONE RELEASE BY MALE *RHYZOPERTHA DOMINICA* (F.) (COLEOPTERA: BOSTRICHIDAE) IN THE LABORATORY WAS AFFECTED BY HOST PLANT, BUT NOT BY BEETLE SIZE

For: Journal of Chemical Ecology

Subject matter: Chemical Ecology

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Pheromone Release by Male *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in the Laboratory was Affected by Host Plant, but not by Beetle Size.

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Abstract

Males of *Rhyzopertha dominica* (F.), the lesser grain borer, produce two aggregation pheromones Dominicalure-1 (DL-1) and Dominicalure-2 (DL-2), which elicit recruitment of conspecifics for mating and to locate food resources. In a laboratory study, pheromone emissions by single males feeding on different host plants was analyzed by gas chromatography in order to compare effects of host plant on pheromone production. Measurement was also made from individual male *R. dominica* feeding on similar host to investigate variation in pheromone production and determine the relationship, if any, between body size and pheromone production in the species. Mean total pheromones (DL-1+DL2) released by male *R. dominica* confined on wheat seeds for 24 hrs was 1,060 ng, at about 20 days of age. There was a drastic reduction in mean total pheromone (DL-1+DL-2) from 1,246 ng to about 60 ng, when males were signaling on plant parts such as seeds especially cereal grains, compared to feeding woody tissues such as elm twigs as food sources. In general, more DL-1 than DL-2 was released across and within plant species. However, there were no interactions between either of the two pheromones (DL-1 or DL-2) and plant species. There was considerable variation in mean quantities of individual pheromones DL-1 or DL-2 and total quantities of pheromone (DL-1+DL-2) released. Proportions of DL-1 in the pheromone blend ranged from 41-61%, and averaged 51%. Regression analyses indicated no relationship between either of the three size indices, body length, pronotum width or fresh body weight, and any pheromone characteristics: quantity of DL-1 or Dl-2 individually, total quantity of DL-1 and DL-2, or the percentage of DL-1 in the pheromone blends. This result suggests that beetle size may have no relationship with pheromone production in *R. dominica*. We concluded that

host plant, rather than beetle body size, significantly affects pheromone release by *R. dominica*.

Keywords. Lesser grain borer, aggregation pheromone, plant species, beetle size

Introduction

THE MALE-PRODUCED AGGREGATION PHEROMONES of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), consist of two unsaturated esters (S)-(+)-1-methylbutyl (*E*)-2-methyl-2-pentenoate, designated dominicalure-1 or DL-1, and (S)-(+)-1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate, designated dominicalure-2 or DL-2 (Khorramshahi and Burkholder 1981, Williams et al. 1981). Both sexes respond strongly in field and laboratory environments to a synthetic blend of DL-1 and DL-2. Despite extensive research efforts to understand the chemical ecology of *R. dominica* (Khorramshahi and Burkholder 1981, Williams et al. 1981, Mayhew 1994, Bashir 2000) recent reviews of the literature (Bashir 2000, Edde 2005) indicated that much remains to be understood on the biology and dynamics of *R. dominica* pheromone production. Because pheromones play a crucial role in mediating aggregation by *R. dominica* in grain storage, a detailed understanding of the pheromone biology of *R. dominica* may aid in elucidating the intricacies of the infestation process. This understanding can then augment research on development of novel management tactics for the pest.

Similar to other insect species that utilize aggregation pheromones (Fadamiro et al. 1998, Borgemeister et al. 1999, Byers 1995, Pureswaran and Borden 2005), *R. dominica* exhibits little to no attraction to host plant volatiles under field conditions (Edde unpublished data), suggesting that only a small proportion of dispersing beetles, if any, would need to employ primary attraction for host finding. Therefore, *R. dominica* that are initial colonizers will maximize fitness if they could produce amounts of aggregation pheromones that are detectable by dispersing beetles. However, studies have shown pheromone output in a variety of insect species may not always be uniform in

absolute quantities and/or in the ratio of pheromone blends (Schlyter and Birgersson 1989, Svensson et al. 1997, Bashir et al. 2003a). Factors that may affect pheromone output in insects include: genetics, behavioral plasticity e.g. reducing pheromone output in the presence of other signalers, diurnal rhythms, body weight, size, age, and host plant quality (Birgersson et al. 1988, Schlyter and Birgersson 1989, Svensson et al. 1997, Bashir et al 2003a, 2003b, Wertheim et al. 2005). Of these factors, insect body weight and body size may be affected by host plant quality, and could have significant influence on life history parameters such as fecundity, successfulness of brood produced, adult mortality, mate recruitment, and overwintering success (Langor et al. 1990, Zvereva 2002, Pureswaran and Borden 2003, Helms and Hunter 2005).

Little is known about how host suitability affects pheromone dynamics in *R. dominica*. A recent report (Bashir et al. 2003b) found that pheromone release rates in *R. dominica* were lowered when male signalers were moved from maize grains, a suitable host, to peanut seeds, an unsuitable host, but release rates were restored when the move was reversed. The workers also reported that ratio of DL-1 to DL-2 was affected by differences in host species. However, their study involved only two plant species, maize, *Zea mais* L. (Gramineae), and peanuts, *Arachis hypogea* L. (Leguminosae). *R. dominica* is reportedly highly polyphagous, and the insect has been recorded breeding on diverse postharvest crops such as legumes, tubers, bulbs, cereals and packaging material made from wood, and on several forest tree seeds (Potter 1935, Wright et al. 1990). Thus, it is necessary to broaden research on pheromone production to several plant species, including suitable and unsuitable hosts. Such studies may facilitate understanding of the nutritional ecology of the species and may aid in identification of useful physical and

chemical cues in plants that may be manipulated to confer advantage against production of attractive signals in *R. dominica*.

Body size is known to influence reproductive success in several species of insects (Thornhill and Alcock 1983) and more specifically, can affect pheromone synthesis (Reid and Roitberg 1995, Pureswaran and Borden 2003, Byers 2005). For example, Byers (2005) observed that quantities of the alarm pheromone (E)-β-farnesene by *Aphis gossypii* (Homoptera: Aphidae) feeding on *Gossypium hirsutum* L. increased in relation to increasing body weight, and variation in individual weights explained about 82% of the variation in alarm pheromone. Pureswaran and Borden (2003) reported that body size was positively correlated with production of anti-aggregation pheromones in male *Dendroctonus ponderosae* (Coleoptera: Scolytidae) paired with females. Working with another scolytid, *Ips pini* (Say), Reid and Roitberg (1995) reported that large males had higher fitness and produced larger offspring than smaller males. Reid and Stamps (1997) hypothesized that female *I. pini* could deduce information about male reproductive fitness from the male-produced pheromone ipsdienol. Information is lacking on the effect of body size on reproductive success, particularly on pheromone emission in *R. dominica*. However, in the related bostrichid, *Prostephanus truncatus* (Horn.), Birkinshaw (1998) found no correlation between male body weight and the attractiveness of the pheromone signals released.

The first objective of this study was to investigate pheromone release by male *R. dominica* fed on different food sources. The second objective was to investigate the effect of beetle size, using beetle weight, overall size and pronotum width as indices of body size, on pheromone output in the species. By quantifying pheromone emission by

individual beetles, we hope to investigate degrees of variation for the quantity of DL-1, quantity of DL-2, total quantity of pheromone produced (DL-1+DL-2), and on ratio of DL-1 in the pheromone blend as affected by adult food source and beetle size.

Materials and Methods

Insect culture. *R. dominica* used in the study were from a beetle colony started from field-collected insects caught in pheromone-baited traps near a grain storage facility in central Oklahoma. The insects were maintained on wheat at 29-30°C and 65% relative humidity in 12-hour light/12-hour dark cycle for about a year before use in the study. Emerged adults were sexed by gently squeezing the abdominal region to extrude the genitalia, which were viewed under a dissecting microscope (Crombie, 1941). Male *R. dominica* used to generate pheromone in the experiments were less than a week old. All beetles used, except when otherwise indicated, were first conditioned by starving them for 24 hrs, after which they were transferred to the plant materials to be tested for pheromone collection.

Pheromone collection method. Male *R. dominica* in all experiments were placed individually in a cylindrical glass aeration chamber measuring 2.75 cm by 7.5 cm containing a food source. The chambers were male and female ground glass joint pieces tapered distally at each end to about 65 mm glass tube that were clamped to a stand and oriented vertically (Fig. 1). Top and bottom openings of the chamber were loosely packed with glass wool to prevent insect escape while allowing airflow. A vacuum pump was used to draw air through 30 ml of water held in a 50 ml side-arm Erlenmeyer flask, the humidified air was drawn through charcoal and Super-Q^{TM} (350 mg, 60-100 mesh)

(Alltech Associate Inc. Deerfield, IL, USA) pre-filters into the aeration arena. Volatiles were collected upwind on a glass column (6 mm internal diameter, and 106 mm long) packed with 300 mg Super-Q^{TM} at an air-flow rate of 300 ml/min. Volatiles were eluted from columns with 2 ml of hexane (Fisher Scientific, HPLC grade). Immediately after elution, 425ng of N-tetradecane (chemical purity 99.5%) was added as an internal standard and the extract was stored at -20°C until analyzed. Aeration was conducted in a room maintained at 29-30°C and 65% relative humidity in 12-hour light/12-hour dark cycle. Twenty aeration devices were available for simultaneous use.

Chemical analyses. Samples were concentrated to 50 µl under a gentle stream of pure N_2 at room temperature before chemical analysis. 1.5 µl of the concentrated sample was analyzed by gas chromatography (Shimadzu GC-17A Ver.3, Shimadzu Corporation, Kyoto, Japan) using a 30 m x 0.25 mm DB-XLB fused silica capillary column (J&W Scientific, Folsom, CA, USA), with helium carrier gas at a flow rate of 1.1 ml/min and flame ionization detector. Injection was made with the purge off, but then opened at 30 sec. The gas chromatography temperature program used was started at 75°C, held for 20 sec, then increased to 120°C at 10°C per minute, and held for 2 minutes, then increased to 160°C at 5°C per minute, held for 2 minutes, and finally increased to 290°C at 15°C and held for 4 minutes. The injector temperature was 230°C and the detector 300°C. Data were processed with a Shimadzu CR 501 integrator (Shimadzu Corporation, Kyoto, Japan). Using the same temperature program, synthetic DL-1 and DL-2, and tetradecane were analyzed to generate retention time of the compounds. The chemical purity of DL-1, DL-2 and N-tetradecane was 95.6, 93.8 and 99.5%, respectively, as determined by our laboratory using gas chromatography. The amounts of DL-1 and DL-2 emitted by *R.*

dominica were calculated by comparing the peak areas with that of the internal standard representing 425 ng in the initial solution. Peak identities of DL-1 and DL-2 in samples were confirmed periodically by gas chromatography-mass spectrometry with reference to authentic standards (Fig. 2, 3).

Experiment 1. Effect of rearing media on pheromone output. A preliminary study was conducted to investigate the effects of larval host plant on pheromone production in *R. dominica*. To achieve this objective, pheromone output by male beetles reared for two generations on seeds of maize or post oak, *Quercus stellata* Wangenh (Fagaceae) (acorns) was measured, when the insects were fed on either their rearing host (i.e. maize or acorn), or on wheat kernels (*Triticum aestivum* L. (Gramineae). Pheromones were collected over a 24-hr period. Based on the outcome of Experiment 1, wheat was used as the rearing media for all insects used in subsequent experiments.

Experiment 2. Pheromone output on different plant tissues. Tissues of seven plant species were used in this study: wheat seeds; peanut seeds; and cowpea, (*Vigna unguiculata* L. Walp (Leguminosae) seeds; potato, *Solanum tuberosum* L. (Solanaceae) tubers; and 1-2 years old terminal branch sections of post oak, *Quercus stellata* Wangenh (Fagaceae); Eastern Red cedar, *Juniperus virginiana* (Cupressaceae); and Slippery elm (*Ulmus rubra* Muhl. (Ulmaceae). In addition, tissues from lateral roots, approximately 30 cm from the tip, of post oak and acorns (matured seeds) were used, yielding nine different plant treatments. Many of the plant species or plant parts used in this study have been suggested as possible hosts to *R. dominica* (Potter 1935, Wright et al. 1990). Wheat seeds were obtained from a batch of freshly harvested hard red winter wheat grown from a local farm near Stillwater, OK. Shelled peanut and cowpea seeds and potato tubers were

obtained from a local grocery retailer. Acorns, cedar, oak and elm twigs were harvested from live trees at a local field site (36°03′N; 097°10′W), which is a naturally self-regenerating forest.

Uniform pellets of host plant materials were fabricated for this experiment. To obtain dried tissues of acorn and potato, the plant materials were cut into cubes (≈7 mm^3), spread thinly on a tray and dried outdoors under natural sunlight. Similarly, twigs from cedar, elm and oak twigs were cut into pieces (≈1cm long), and sun-dried. To ensure that the plant materials used as food sources were of similar particle size and density, the plant materials were pulverized using a mechanical grinder and sifted using a # 21 sieve (Seedburo Equipment Co., Chicago, IL.). Pulverized materials were made into pellets containing a mixture of plant material, agar and cellulose in the ratio 4:2:1(plant materials: agar: cellulose) by weight. Pellets were prepared by adding warm water to agar in a glass flask and then thoroughly mixing with cellulose, followed by plant materials. About 50 ml of water was used for preparing the mixtures. Experimental pellets were a mixture of agar and cellulose in the ratio 5:2 (agar: cellulose). The ensuing paste mass from the mixtures were firmly pressed and extruded through holes (1.5 cm inner diameter and 0.5 cm deep) drilled in a PVC sheet. Pellets were spread thinly in a tray on a laboratory bench to air dry. Moisture contents of the pellets were equilibrated to about 15.3% at the start of the experiment. Moisture content equilibration was achieved by placing Petri dishes containing the pellets in humidifiers containing saturated sodium chloride solution for six days prior to start of the experiment. The weight of the experimental pellets ranged between 0.10 and 15.0 g, and a mean of 0.13 g.

The influence of different food treatments on pheromone output was investigated by introducing one starved male beetle on two pellets of each food treatment in an aeration chamber. Pheromones were collected over a 24-hr period.

Experiment 3. Pheromone output in relation to beetle size. Beetle body length, pronotum width and fresh body weight, were used as indices of beetle size. Body length was measured from the anterior margin of the pronotum to posterior margin of the elytra to the nearest 0.01 mm, using a dissecting microscope fitted with an ocular micrometer. Pronotum width was measured from lateral right to lateral left margins of the middle of pronotum. A preliminary experiment conducted on longevity of starved *R. dominica*, under similar temperature and humidity regimes as in Experiment 1, indicated that male *R. dominica* can survive for 11 days without food and water. Mean survival time was 6 days. Therefore, beetles used in this experiment were starved for 6 days in order to stabilize beetle body weight and feeding status. Fresh body weight of the insects before and at the end of the 6 days starvation period was determined to the nearest 0.001 mg, using a Sartorius electronic microbalance type M3P (Satorius Instruments, McGaw Park, IL). Food-deprived beetles were individually refed *ad libitum* on 1 g of crushed wheat kernels in pheromone collection chambers from which pheromones were collected every 24 hours for six consecutive days. Pheromones were collected from 30 food-deprived beetles. Unstarved males were used as controls. The result showed that that the quantities of pheromones produced by starved beetles on day 5 of the refeeding period became stabilized, and were similar to quantities produced by unstarved beetles. However, data on pheromones collected on the sixth day from the food-deprived insects were used to investigate the effect of beetle size on pheromone output. The fresh body weight of the

insects at the end of pheromone collection exercise (day 6) were determined. Details on beetle longevity and effect of starvation on pheromone production in *R. dominica* will be reported elsewhere.

Statistical analysis. All data were analyzed using PC SAS version 8 (SAS Institute, 2001). Data in Experiment 1-3 were analyzed as a two-factor experiment using the PROC MIXED in which the main factors were plant species and pheromones (DL-1 and DL-2). PROC TTEST was used to analyze differences between mean quantities of DL-1 and DL-2 released within treatments in Experiments 1and 2. Linear regression analysis (PROC REG) was performed on scatter plots of beetle weight, body length and pronotum width to determine relationship between beetle size and pheromone production. Coefficient of variation (Zar 1999) was used to determine individual variation for pheromones emitted in Experiments 2 and 3, and as a measure of variation in weight loss among starved beetles in Experiment 3.

Results

Effect of rearing media on pheromone output. A two-factor analysis of variance indicated no interaction between pheromone (DL-1 or DL-2) and plant species ($F = 0.51$; df = 5, 148; $P = 0.7663$) in the pheromone blend released. Although, the absolute quantities of DL-1 and DL-2 released by males differed significantly ($F = 3.96$; df = 5, 148; $P < 0.01$) among plant species, there was no significant difference in the quantities of DL-1 and DL-2 emitted when beetles were on wheat or maize (two suitable host plants), irrespective of the plant species on which the beetles were reared (Table 1). Results further indicate that the main effect of pheromones released was significant ($F =$

30.13; df = 5, 148; $P < 0.001$), such that more DL-1 than DL-2 was released among the plant species. Likewise, output of DL-1 was higher than DL-2 within plant species, except in the treatments where males were reared on wheat, but aerated on maize, and for the two treatments involving acorns. Emission of both pheromones was not affected by rearing host in any case, and was reduced only significantly when acorn was the feeding host for aeration (Table 1).

Pheromone output on different plant tissues. All male *R. dominica* were observed feeding as indicated by feeding dust produced, and released aggregation pheromones on all pellets (Fig. 4). Males feeding on pellets made from seeds were observed to have higher boring rates as indicated by quantities of frass produced, compared to those from woody tissues. Mean quantities of total pheromones (DL-1+DL-2) released differed significantly ($F = 39.22$; df = 9, 90; $P < 0.001$) among food sources. Beetles feeding on wheat pellets released the highest amount (1,246.3 ng) of total pheromones (DL-1+DL-2), and was significantly different from total pheromones released on other treatments (Table 2). The lowest pheromone output (59.96 ng) was observed on elm pellets, and was similar to pheromone emitted by beetles feeding on agar pellets, and pellets made from twig or root of oak, cedar twig, potato tubers and peanut (Table 2).

The results also revealed that the mean quantities of DL-1 and DL-2 released differed significantly among plant species ($F = 6.51$; df = 1, 180; $P < 0.05$). In general, more DL-1 than DL-2 was released. A two-factor analysis of variance indicated no interaction between pheromone (DL-1 or DL-2) and plant species ($F = 1.18$; df = 9, 180; $P = 0.06$) in pheromone blend produced. Similarly, mean quantities of either DL-1 or

DL-2 were not significantly different within plant species, except on wheat and acorn (Table 2).

Pheromone output in relation to beetle size. Body length and pronotum width ranged between 2.35 and 0.73 mm and 2.79 and 0.88 mm, respectively. Mean body length and pronotum width was 2.59 and 0.84 mm, respectively. Results of the analysis of coefficient of variation (CV) showed that the relative variation among beetle fresh body weight, body length and pronotum width was 8.21, 3.74 and 5.15%, respectively.

Every male *R. dominica* used in the study released aggregation pheromones; however, there was considerable variation in mean quantities of individual pheromones, DL-1 or DL-2, and total quantities of pheromone (DL-1+DL-2) released (Table 3). Mean total pheromones (DL-1+DL2) released by male *R. dominica* confined on wheat for 24 hrs was 1,060 ng, at about 20 days of age. The coefficient of variation (CV) showed that the amount of variation in the quantity of DL-1, DL-2, and DL-1+DL-2 was 31.3, 24.3 and 26%, respectiviely. The amount of variation in the proportion of DL-1 in the pheromone blend, however, was smaller (about 10%) than for absolute quantities of the pheromone. Regression analyses (Table 4) indicated no relationship between length, pronotum width and weight and any pheromone characteristics: quantity of DL-1, Dl-2, total quantity (DL-1+DL-2), and the percentage of DL-1 in the pheromone blend.

Discussion

Results from Experiment 2 demonstrated that male *R. dominica* feeding on plant parts such as seeds, especially cereal grains, generated higher total pheromone released (DL-1+DL-2) than on woody tissues or non-cereal hosts.

Although we did not attempt to characterize the biochemical properties of the different plant species used as food sources in the present study we suspect that interspecific differences in nutritional quality among plant species might largely be responsible for the observed variations in pheromone output by male *R. dominica*. Plant seeds are high in readily digestible sugars, amino acids, phospholipids etc and low in fiber compared with the high levels of poorly digestible compounds such as cellulose, lignin and tannins inherent in woody tissues (van Etten et al. 1967). Presumably, males feeding on pellets made from seeds (e.g. wheat) might have had better access to nutrients needed to initiate and/or sustain pheromone production, and consequently released larger amounts of pheromones than those feeding on woody tissues. In addition, many plant seeds are rich in non-nutritive constituent such as glycosides, alkaloids, terpenoids, etc, many of which may serve as phagostimulants for phytophagous insects (Bernays and Chapman 1994). Males feeding on pellets made from seeds had higher boring rate as indicated by quantities of frass produced, compared to those from woody tissues. We do not know if increased boring activities correlates with feeding, however, previous studies have positively correlated boring rate with pheromone emission rate in *R. dominica* (Bashir et al. 2003a), and in several bark beetles including *Ips paraconfusus* Lanier (Elkinton et al. 1980). Additionally, Mayhew (1994) clearly showed that feeding activity was required for pheromone output in *R. dominica*, and that pheromone production was significantly decreased when the amount of food per male, or the nutritional quality of the food, was decreased.

In a previous study, Edde and Phillips (unpublished) found that traps baited with live male *R. dominica* feeding on wheat were highly attractive to dispersing conspecifics,

moderately attractive when feeding on acorn, potato and cowpea, and had low, but biologically significant attraction when feeding on peanut. Interestingly, that ranking parallels observations on reproductive success of the insect on these plant species (Edde and Phillips unpublished) and pheromone release reported in the current study. Males feeding on wheat most likely released the higher quantities of pheromones (DL-1+DL-2) relative to beetles feeding on less suitable plant species in our field experiments, and this suggestion is confirmed directly by results in the present study. For example, there was a drastic reduction in mean total pheromone (DL-1+DL-2) released from 1246 ng when males were fed on wheat to about 60 ng when fed on elm twigs. Collectively, the observations by Edde and Phillips (unpublished) and results from the current study (Table 2 and Fig. 4) further suggest that the total quantities of pheromone (DL-1+DL-2) released, rather than modifications of ratios in pheromone blend, as suggested by Bashir (2000), may be the key aspect of pheromonal signals determining the success of aggregation behavior in *R. dominica.* Therefore, one way to analyze and understand the influence of host quality on pheromone signaling in *R. dominica*, and consequently its ability to aggregate conspecifics, may be to measure absolute quantities of total pheromone signals (DL-1+DL-2) released by the insect. This suggestion is supported by the fact that we did not observe any interactions between either of the pheromones (DL-1 or DL-2) and plant species in this study. In addition, previous trapping studies using synthetic lures DL-1 or DL-2 or their mixture found individual pheromone or their mixture were equally attractive to both sexes, but attractancy increased with dosage (Cogburn et al. 1984, Burkholder and Ma 1985, Edde unpublished data), suggesting that one of the pheromones may be redundant (Linn et al. 1984, McBrien et al. 2002).

Furthermore, it is well established that at natural release rates, an increase in aggregation pheromones release rate increases the attractive distance and/or temporal pattern of odor exposure of pheromones (Baker and Kuenen 1982, Roelofs 1978, Byers 1995), thus increasing the chances of responders locating the signaler.

Mean total pheromones (DL-1+DL2) released by male *R. dominica* confined on wheat for 24 hrs was 1,060 ng, at about 20 days of age. This was slightly lower than the 1,300 ng mean daily production of DL-1 +DL2 reported by Mayhew (1994) for one male *R. dominica* confined on wheat after 12 days of feeding. However, our results and that by Mayhew (1994) were much lower than mean daily release of 2,300 ng mean daily production of DL-1+DL-2 reported by Bashir et al. (2003a). Although, the later researchers used maize as food sources in their study, the results presented in Table 1 indicated that pheromone released by male *R. dominica* feeding on wheat or maize was similar, thus differences in food sources could not have accounted for the higher pheromones output by Bashir et al. (2003a). It is likely, as previously suggested by Bashir et al. (2003a), that differences in the strain of insects used in three studies may be responsible for differences in pheromone output observed. The ratio of DL-1 to DL-2 observed in the present study (Table 3), however, was similar to the range found by Mayhew (1994) and Bashir et al. (2003a).

Every male *R. dominica* used in Experiment 3 generated aggregation pheromones. However, similar to observations on pheromone output on different food sources, beetles feeding on the same host varied in the absolute amount (DL-1+DL-2), and in the ratio of DL-1 to DL-2 (Table 3). Similar to findings by Bashir et al. (2003a), the amount of variation observed for absolute quantity of DL-1 (31.3%) and DL-2 (24.3%) was greater

than for proportion of DL-1 (9.99%) in the blend. The amount of variation of DL-1 in the pheromone blend in the current study was, however, less variable than suggested by Bashir et al. (2003a). Intermale variation in pheromone output has been suggested for the bostrichid *P. truncatus* (Hodges et al. 2002), and is a common phenomenon in the sex pheromones of several species of Lepidoptera (Schlyter and Birgersson 1989, Kou and Chow 1991, Svensson et al. 1997), and in aggregation pheromones of several bark beetles (Schlyter and Birgersson 1989, Pureswaran and Borden 2003).

Results from this study suggested that aggregation pheromone production in *R. dominica* is not affected by beetle size. This is in contrast to findings in other insect species. Working with *Aphis gossypii* (Homoptera: Aphidae), Byers (2005) observed that quantities of the alarm pheromone (E)-β-farnesene feeding on *Gossypium hirsutum* L. increased in relation to increasing body weight. Similarly, Pureswaran and Borden (2003) found significant relationships between body size and the release of anti-aggregation pheromones in *Dendroctonus ponderosae* (Coleoptera: Scolytidae) males paired with females. The lack of correlation between body size and pheromone output in *R. dominica* reported in this study parallel findings in other wood boring beetles. For example, by Birgersson et al. (1988) found no correlation between body size and output of *cis*-verbenol, one of the aggregation pheromones of *I. typographus*. Despite a correlation with anti-aggregation pheromones, Pureswaran and Borden (2003) observed a lack of correlation between body size and output of attractive aggregation pheromones in either sex of *D. ponderosae* emerging from natural attacked host. Similarly, Birkinshaw (1998) did not find any linkage between the weight of males and the attractiveness of the pheromone signals released by *P. truncatus*. Body size varies slightly in *R. dominica*. It is

more probable that variations in pheromone output between male beetles feeding on similar host, and under the same environmental conditions, and certainly among those on different hosts or conspecific host of different quality, may be attributed to differences between the individuals (Bashir et al. 2003a, Birgersson et al. 1988). In a related study, Svensson et al. (1997) found that a major part of individual female sex pheromone variation in turnip moth *Agrotis segetum* (Lepidoptera: Noctuidae) was explained by variation between individuals and not within individuals. Variation among male *R. dominica* in pheromone emission may have significant evolutionary consequences because it is the male, presumably, that locate good hosts then signal that fact with their pheromones. Thus, males that are highly attractive may easily be located by females, and therefore, have higher mating success than males that are moderately attractive.

The data presented here demonstrated that *R. dominica* is able to produce pheromone on non-cereal crops such as cowpeas and dried potato tubers, and on non-agricultural hosts. Previous observations (Wright et al. 1990, Edde and Phillips unpublished) have indicated the ability of the insect to reproduce on some of the non-cereal and wild hosts investigated in the present study. This is quite interesting, and is an indication that non-cereal and/or nonagricultural habitats could act as alternative hosts for the pest in the absent of preferred host like wheat and maize. Male *R. dominica* feeding on plant parts such as seeds, especially cereal grains, generated higher total pheromone output (DL-1+DL-2) than on woody tissues or non-cereal hosts, however, there was lack of correlation between body size and pheromone output in *R. dominica*.

Acknowledgement.

The USDA CSREES (Risk Avoidance and Mitigation Program) under Agreement No 2000-05385 and the Oklahoma State University Integrated Pest Management Mini-Grant Program provided financial support for this study.

References

Baker, T. C., and L. P. S. Kuenen. 1982. Pheromone source location by flying moth: a supplementary non-anemotactic mechanism. Science. 216: 424-427.

Bashir, T. 2000. Pheromone communication and host finding behavior of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) Ph.D. thesis, University of Greenwich, U.K.

Bashir, T., L. A. Birkinshaw, D. Farmn, D. R. Hall, and R. J. Hodges. 2003a. Pheromone release by *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in the laboratory: daily rhythm, inter-male variation and association with body weight and/or boring activity. J. Stored Prod. Res. 39: 159-169.

Bashir, T., R. J. Hodges, L. A. Birkinshaw, D. R. Hall, and D. I. Farman. 2003b. Phenotypic plasticity of *Rhyzopertha dominica* pheromone signaling: The effects of different hosts and presence of conspecific females on male produced aggregation pheromone. J. Chem. Ecol. 29: 945-959.

Bernays, E. A., and R. F. Chapman. 1994. Host-plant selection by phytophagous insects. Chapman and Hall, New York, NY.

Birgersson, G. 1989. Host tree resistance influencing pheromone production in Ips typographus (Coleoptera: Scolytidae). Holarct. Ecol. 451-456.

Birgersson, G., F. Schlyter, G. Bergstrom, and J. Lőfqvist. 1988. Individual variation in aggregation pheromone content of the bark beetle, *Ips typographus*. J. Chem. Ecol. 14: 1737-1761.

Birkinshaw, L. A. 1998. Mate choice in Prostephanus truncatus (Coleoptera: Bostrichidae): The role of male-produced aggregation pheromones. PhD. thesis. University of Leicester, Leicester, UK.

Borgemeister, C., K. Schaefer, G. Goergen, S. Awande, M. Setamou, and H. -M. Poehling. 1999. Host-finding behavior of *Dinoderus bifoveolatus* (Coleoptera: Bostrichidae), an important pest of stored cassava: The role of plant volatiles and odors of conspecifics. Ann. Entomol. Soc. Am. 92: 766-771.

Burkholder, W.E and M. Ma. 1985. Pheromones for Monitoring and Control of Stored-Product Insect. Ann. Rev. Entomol. 30: 257-272.

Byers, J. A. **1995**. Host tree chemistry affecting colonization in bark beetles, pp 154-213. *In* R. T. Cardé, and W. J. Bell (eds.), Chemical ecology of insect 2. Chapman and Hall, New York.

Byers, J. A. 2005. A cost of alarm pheromone production in cotton aphids, *Aphis gossypii*. Naturwissenschaften. 92: 69-72.

Cogburn, R. R., W. E. Burkholder, and H. J. Williams. 1984. Field test with aggregation pheromone of the lesser grain borer (Coleoptera: Bostrichidae) [*Rhyzopertha dominica*, pest of stored grain]. Environ. Entomol. 13: 162-166.

Crombie, A. C. 1941. On oviposition, olfactory conditioning and host selection in *Rhyzopertha dominica* Fab. (Insecta, Coleoptera). J. Exp. Biol. 18: 62-79.

Edde, P. A. 2005. Studies with the aggregation pheromones of *Rhyzopertha dominica* (Coleoptera: Bostrichidae): Habitat affinities, seasonal flight activity, and pheromone-mediated host selection behavior. Ph.D. dissertation. Oklahoma State University, Stillwater, OK, USA.

Elkinton, J.S., D. I. Wood, and L. B. Hendry. 1980. Pheromone production by the bark beetle, *Ips paraconfusus*, in the non-host, white fir. J. Chem. Ecol. 6: 979-987.

Fadamiro, H. Y., I. Gudrups, and R. I. Hodges. 1998. Upwind flight of *Prostephanus truncatus* is mediated by aggregation pheromone but not food volatiles. J. Stored Prod. Res. 34: 151-158.

Helms, S.E. and M.D. Hunter. 2005. Variation in plant quality and the population dynamics of herbivores: there is nothing average about aphids. Oecologia (in press).

Hodges, R., J., L.A. Birkinshaw, D. I. Farman, and D. R. Hall. 2002. Intermale variation in aggregation pheromone release in *Prostephanus truncatus*. J. Chem. Ecol. 28: 1665-1674.

Khorramshahi, A., and W. E. Burkholder. 1981. Behavior of the lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J. Chem. Ecol. 7: 33-38.

Kou, R., and Y. S. Chow. 1991. Individual variation in sex pheromone of smaller Tea-tortrix moth *Adoxophyses* sp. Lepidoptera: Tortricidae. J. Chem. Ecol. 17: 1917-1924.

Langor, D. W., J. R. Spencer, and G. R. Pohl. 1990. Host effects on fecundity and reproductive success of *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). Evol. 44: 609-618.

Linn, C.E., L. B. Bjostad, J.W. Du, and W. L. Roelofs. 1984. Redundancy in a chemical signal: behavioral responses of male *Trichoplusia ni* to a 6-component sex pheromone blend. J. Chem. Ecol. 10: 1635-1658.

Mayhew, T. J. 1994. Pheromone production and behavioral response to pheromones in the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). MS thesis, University of Wisconsin-Madison, USA.

McBrien, H. L., J. G. Millar, R. E. Rice, J. S. McElfresh, E. Cullen, and F. G. Zalom. 2002. Sex attractant pheromone of the red-shouldered stink bug *Thyanta*

pallidovirens: a pheromone blend with multiple redundant components. J. Chem. Ecol. 28:1797-818.

Potter, C. 1935. The biology and distribution of *Rhyzopertha dominica* (Fab.). Trans. Royal Entomol. Soc. 83: 449-482.

Pureswaran, D. S., and J. H. Borden. 2005. Primary attraction and kairomonal host discrimination in three species of *Dendroctonus* (Coleoptera: Scolytidae). Agric. For. Entomol. 16: 765-782.

Pureswaran, D. S., and J. H. Borden. 2003. Is bigger better? Size and pheromone production in the mountain pine beetle *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). J. Insect Behav. 16: 765-782.

Reid, M. L., and B. J. Roitberg. 1995. Effects of body size on investment in individual broods by male pine engravers (Coleoptera: Scolytidae). Can. J. Zool. 73: 1396-1401.

Reid, R. W., and J. A. Stamps. 1997. Female mate choice tactics in a resource-based mating system: Field tests of alternative methods. Am. Nat. 150: 198-121.

Roelofs, W. L. 1978. Threshold hypothesis for pheromone perception. J. Chem. Ecol. 4: 685-699.

SAS Institute. 2001. SAS/STAT User's Guide, version 8, SAS Institute Inc., Cary, NC.

Schlyter, F., and G. Birgersson. 1989. Individual variation in bark beetles and moth pheromones – a comparison and an evolutionary background. Holartic Ecol. 12: 457-465.

Svensson, M. G. E., M. Bengtsson, and J. Lőfqvist. 1997. Individual variation and repeatability of sex pheromone emission of female turnip moths *Agrotis segetum*. J. Chem. Ecol. 23: 1833-1850.

Thornhill, R., and J. Alcock. 1983. The evolution of insect mating system. Harvard University Press, Massachusetts.

Van Etten, C. H., W. F. Kwolek, J. E. Peters, and A. S. Barclay. 1967. Plant seeds as protein sources for food or feed. Evaluation based on amino acid composition of 379 species. J. Agric. Food Chem. 15:1077-89.

Williams, H. J., R. M. Silverstein, W. E. Burkholder, and A. Khorramshahi. 1981. Dominicalure 1 and 2: components of aggregation pheromone from male lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). J. Chem. Ecol. 7: 759-781.

Wertheim, B., J. Marchais, L. E. M. Vet, and M. Dicke. 2005. Pheromone-mediated aggregation in non-social arthropods: an evolutionary ecological perspective. Ann. Rev. Entomol. 321-346.

Wright, V. F., E. E. Fleming, and D. Post. 1990. Survival of *Rhyzopertha dominica* (Coleoptera, Bostrichidae) on fruits and seeds collected from woodrat nests in Kansas. J. Kans. Entomol. Soc. 63*:* 344-347.

Zar, J. H. 1999. Biostatistical analysis. Prentice-Hall Inc., Englewood Cliffs, NJ.

Zvereva, E. L. 2002. Effects of host plant quality on overwintering success of the leaf beetle *Chrysomela lapponica* (Coleoptera: Chrysomelidae). Eur. J. Entomol. 99: 189-195.

Table 1. Output of Dominicalure-1 (DL-1) and Dominicalure-2 (DL-2) by *R. dominica* (N = 15) (Mean ± SE) for 24 hrs in collection of volatiles in headspace of feeding insects reared on wheat, maize or acorns

Treatment		DL-1 (ng)	DL-2 (ng)	Comparing DL-1 to DL-2
Rearing host	Feeding host			
Maize	Wheat	711.2±62.8 A	420.7±35.8 A	$P = 0.0002$
Maize	Maize	632.5±46.3 A	436.0±32.1 A	$P = 0.0101$
Wheat	Wheat	719.9±57.8 A	490.5±60.1 A	$P = 0.0028$
Wheat	Maize	678.7±71.7 A	571.2±45.0 A	$P = 0.1548$
Acorn	Wheat	688.1±65.5 A	502.7±47.9 A	$P = 0.0709$
Acorn	Acorn	460.0±98.2 B	261.3±65.8 B	$P = 0.0539$

Means within the same column with the same letter are not significantly different at 0.05 level of significance; ng = nanogram

Table 2. Output of Dominicalure-1 (DL-1) and Dominicalure-2 (DL-2) by male *R. dominica* (N = 10) (mean ± SE) for 24 hrs in collection of volatiles in headspace of insects feeding on different plant species.

Plant species	DL-1	DL-2	comparing DL1 to DL2
Wheat	700.3±40.4 A	545.7±51.9 A	$P = 0.0023$
Cowpea	442.6±60.7 B	370.0±46.3 B	$P = 0.1478$
Acorn	372.3±65.0 B	202.6±36.9 C	$P = 0.0008$
Potato	124.9±18.9 C	133.8±18.1 CD	$P = 0.8589$
Peanut	66.2±34.6 C	43.8±12.1 D	$P = 0.6543$
Cedar	37.9±5.9 C	42.4±7.5 D	$P = 0.9274$
A-Root	37.0±5.5 C	38.4±5.8 D	$P = 0.9766$
A-Twig	31.2±3.8 C	33.8±4.7 D	$P = 0.9597$
Elm	29.5±2.9 C	30.5±3.6 D	$P = 0.9836$
Agar	32.0±2.8 C	29.6±2.2 D	$P = 0.9614$

A-Root – Oak root, A-Twig = Oak stem, Acorn = Oak seed,
Two means within the same column with the same letter are not significantly different at 0.05 level of significance

Table 3. Minimum, maximum, mean quantities and coefficient of variation of Dominicalure-1 (DL-1) and Dominicalure-2 (DL-2) and total quantity of DL-1+DL2 emitted by male *R. dominica* (N = 30) (Mean ± SE) for 24 hrs in collection of volatiles in headspace of feeding insects.

Pheromone	Minimum value	Maximum value	Mean value	CV[1]
DL-1 (ng)	320.49	978.24	549.47	31.30
DL-2 (ng)	302.31	725.83	514.53	24.30
DL1+DL-2 (ng)	622.80	1699.36	1064.00	25.89
Proportion of DL-1 in pheromone blend (%)	0.41	0.61	0.51	9.99

Coefficient of variation.

Table 4. Relation between size and pheromone output by male *R. dominica* (N = 30) placed on wheat for 24 hrs in collection of volatiles in headspace of feeding insects.

Pheromone	Size index	Regression coefficient	R^2	*P*-value
DL-1	Length	y = -300.24x + 1338.8	0.0449	0.2612
	Weight	y = 76.547x + 460.17	0.0024	0.7987
	Pronotum width	y = -404.64x + 880.32	0.0054	0.7003
DL-2	Length	y = -40.783x + 621.75	0.0016	0.8355
	Weight	y = 128.9x + 364.17	0.0127	0.5535
	Pronotum width	y = -728.79x + 1110.4	0.033	0.3369
DL-1 + DL-2	Length	y = -341.02x + 1960.5	0.0226	0.4282
	Weight	y = 205.45x + 824.35	0.0066	0.6687
	Pronotum width	y = -1133.4x + 1490.8	0.016	0.4997
Proportion of DL-1 in pheromone blend	Length	y = -0.1263x + 0.8442	0.0897	0.1080
	Weight	y = -0.039x + 0.5577	0.0069	0.6618
	Pronotum width	y = 0.1307x + 0.4053	0.0063	0.6761

Figure Legend

Fig. 1. Pheromone collection apparatus.

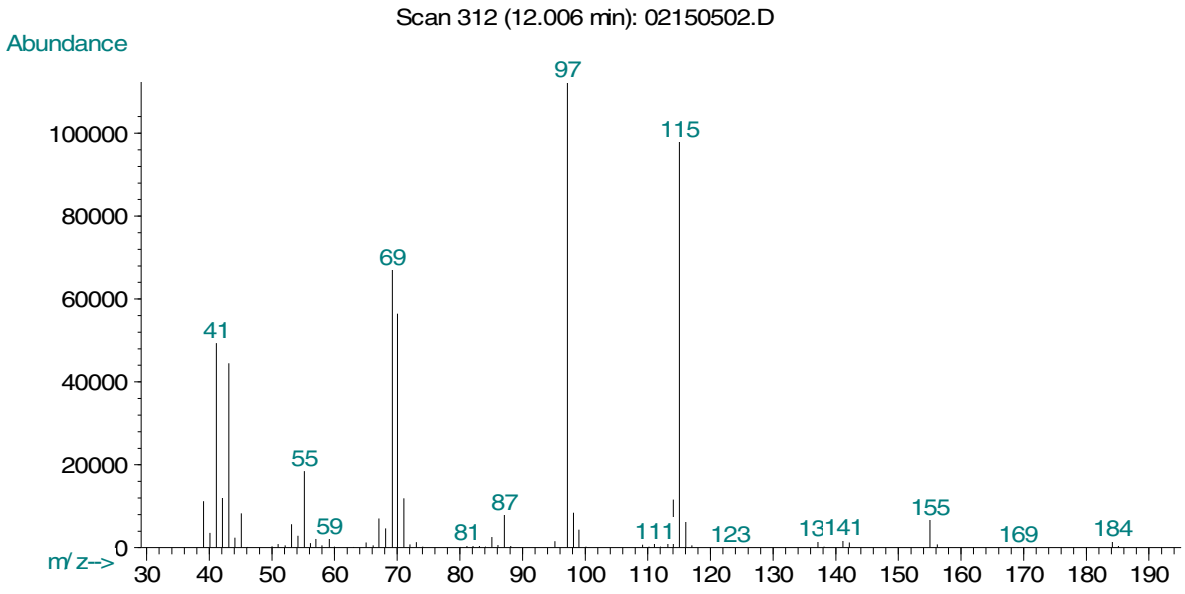
Fig. 2. Mass Spectra of Dominicalure-1 (DL-1).

Fig. 3. Mass Spectra of Dominicalure-2 (DL-2).

Fig. 4. Mean combined quantities of the aggregation pheromones Dominicalure-1 (DL-1) plus Dominicalure-2 (DL-2) released by one male R. dominica in 24 hours period on different food substrates. Bars followed by same letters are not significantly different ($\alpha = 0.05$).

Fig. 1

Fig. 2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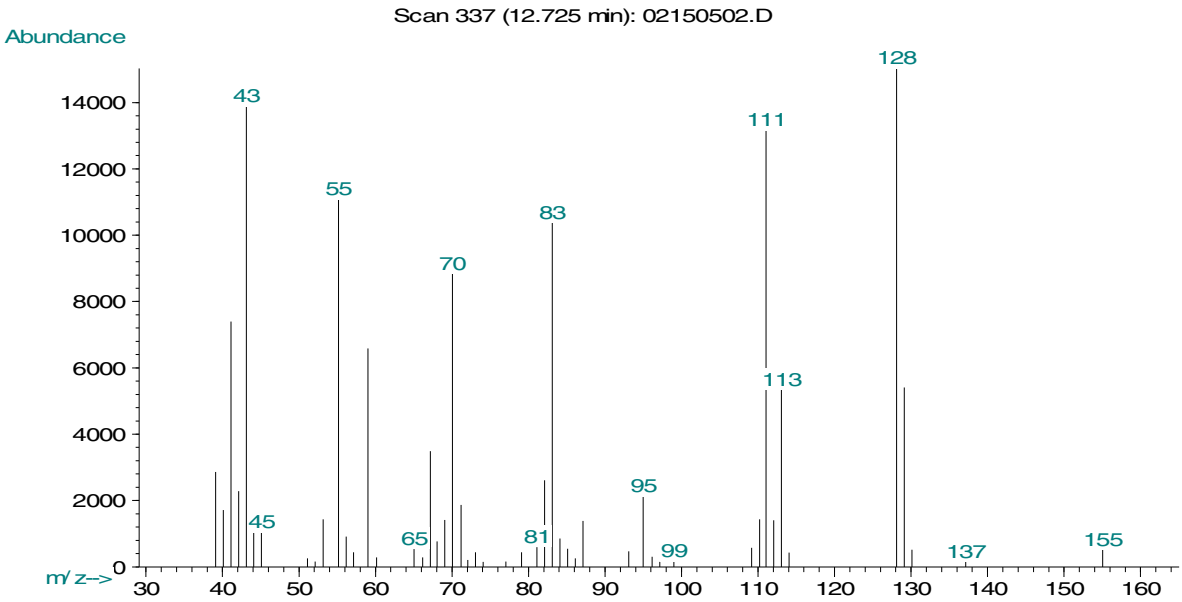
Abundance
m/z-->
97
115
69
41
55
59
87
81
111
123
131
141
155
169
184
100000
80000
60000
40000
20000
0
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190

Fig. 3.


Scan 337 (12.725 min): 02150502.D
Abundance
m/z-->
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12000
10000
8000
6000
4000
2000
0
30
40
50
60
70
80
90
100
110
120
130
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150
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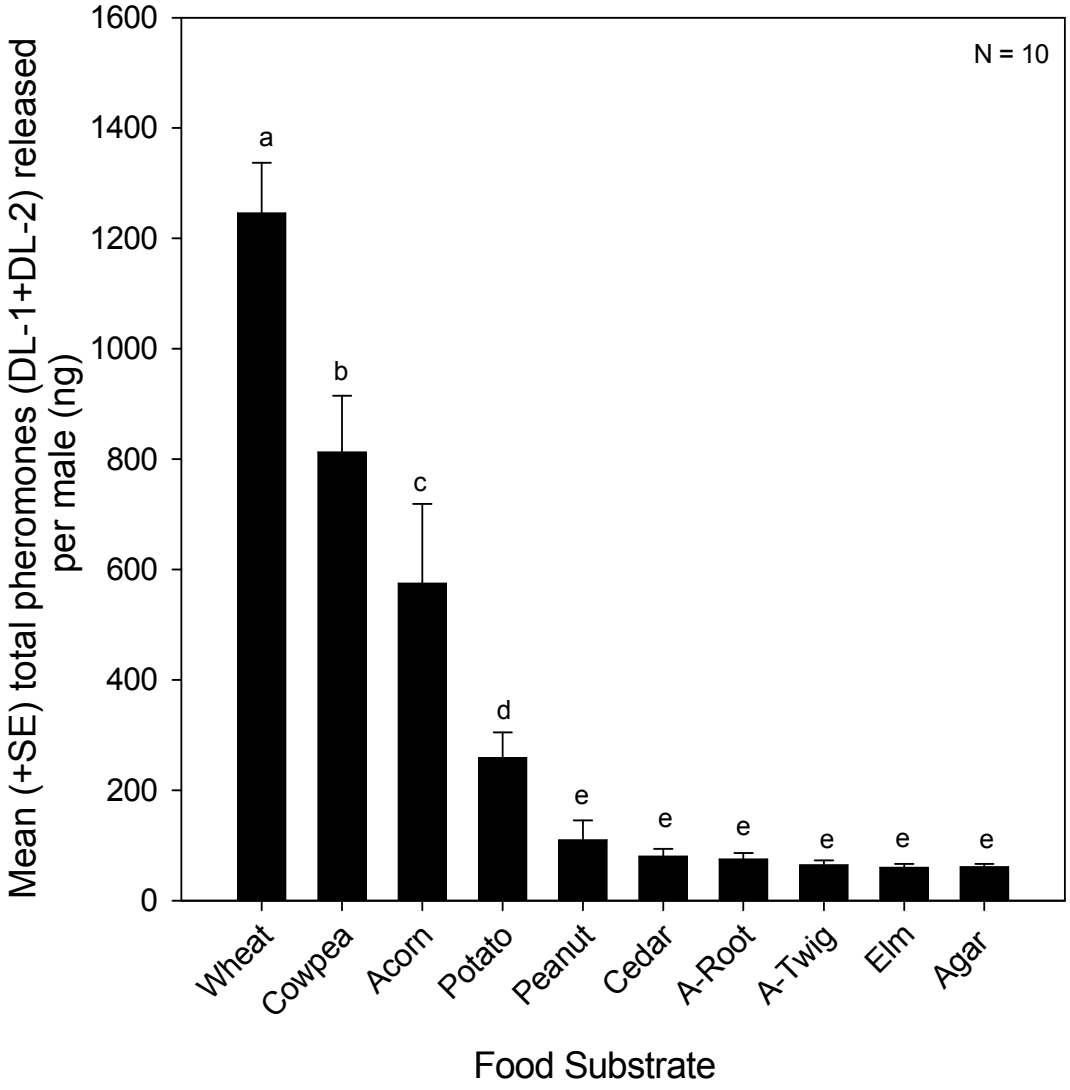
43
55
70
83
111
128
113
95
45
65
81
99
137
155

Fig. 4


Mean (+SE) total pheromones (DL-1+DL-2) released per male (ng)
1600
1400
1200
1000
800
600
400
200
0
N = 10
a
b
c
d
e
e
e
e
e
e
Wheat
Cowpea
Acorn
Potato
Peanut
Cedar
A-Root
A-Twig
Elm
Agar
Food Substrate

CHAPTER VII

SUMMARY

Male *R. dominica* produce two aggregation pheromones, Dominicalure-1 (DL-1) and Dominicalure-2 (DL-2), which elicit recruitment of conspecifics for mating and locating food resources. Both sexes respond strongly in field and laboratory environments to a synthetic blend of DL-1 and DL-2. Despite extensive research efforts to understand the chemical ecology of *R. dominica*, a recent review of the literature (Bashir 2000) indicated that much remains to be understood on the biology and dynamics of *R. dominica* pheromone production. Because pheromones play a crucial role in mediating aggregation by *R. dominica* in grain storage, a detailed understanding of the pheromone biology of *R. dominica* may aid in elucidating the intricacies of the infestation process. This understanding can then augment research on development of novel management tactics for this pest.

Two approaches were adopted in this thesis to elucidate the ecology of host finding in *R. dominica*. The first part of this dissertation investigated seasonal abundance and flight activity patterns of the insect in different habitats in central Oklahoma using synthetic pheromones in monitoring traps. The second part of the study investigated the influence of host plant on reproductive fitness, pheromone production and host finding by *R. dominica.* Major findings from the study are summarized below.

R. dominica populations were greater near grain storage facilities than in forests or in open fields. This ranking apparently reflects the relative ability of these habitats to sustain *R. dominica* populations. The presence of wheat, a primary host plant, in the grain bins during the study may have either increased attraction to those locations, or served as an initial source of beetles. On the other hand, absence of readily available food sources in open field habitats, and relatively long distance of these traps from populations of *R.*

dominica infested grain, might be responsible for the lower numbers of beetles in open fields. The source of *R. dominica* attacking newly stored grain remains unknown. However, we cannot rule out the possibility that beetles trapped in wooded areas (current study) originated from host material other than stored grain. There are anecdotal reports of *R. dominica* tunneling in various tree species in the wild (Potter 1935; Linsley 1944; Mathew 1987).

Seasonal flight activity patterns were similar between habitats; however, in two of the three years during the study, flight activity generally began at least 1-2 weeks earlier in forest sites as opposed to grain elevators. *R. dominica* were most active during the warmer part of the year. No *R. dominica* were trapped from December through February. In general, flight activity of *R. dominica* in central Oklahoma seemed to be tri-modal for each year of this study such that peaks of trap captures occurred in May, Sept and early October. Synchrony of *R. dominica* flight activity patterns between forest and grain storage probably reflect interdependency between the two habitats in sustaining *R. dominica* populations. Additional studies are needed on the dispersal behavior and the effects of habitat quality and interactions between *R. dominica* metapopulations.

Investigations of the behavioral responses of adult *R. dominica* showed that both sexes were able to respond to plant volatiles under short range conditions (walking bioassay), but the attraction was strongest to plant species most suitable for beetle development. Host volatiles alone, however, failed to attract dispersing *R. dominica* under field conditions. In contrast, beetles responded strongly to traps baited with infested plant materials, suggesting a short-range effect in the role of primary attraction in host-finding behavior of *R. dominica*. It is likely that only a small proportion of

dispersing *R. dominica*, if any, would need to employ primary attraction for host finding, because conspecifics exhibit strong orientation to aggregation pheromones released by male beetles. Male-produced aggregation pheromones, therefore, may be considered the most important stimuli for host plant location in *R. dominica*, and may play a crucial role in subsequent aggregation by conspecific males and females. The phenomenon of secondary attraction, i.e. orientation to aggregation pheromones, has been suggested for other stored product bostrichid pests *P. truncatus* and *Dinoderus bifoveolatus* (Fadamiro et al. 1998, Borgemeister et al. 1999), and is very common among wood-boring insects that have strong aggregation pheromones (Byers 1995, Pureswaran and Borden 2005). These species exhibit weak or no attraction to host plant volatiles under field conditions (Fadamiro et al. 1998, Byers 1995, Pureswaran and Borden 2005).

Studies on responses of conspecific *R. dominica* to naturally produced pheromones showed that traps baited with live males feeding on wheat were highly attractive to dispersing conspecifics, moderately attractive when feeding on acorn, potato and cowpea, and had low, but biologically significant attraction when feeding on peanut. This ranking parallels observations on reproductive fitness (Chapter V) and pheromone release by *R. dominica* on these plant species (Chapter VI). Results from chapter VI also showed that males feeding on plant seeds (e.g. wheat) released higher quantities of pheromones (DL-1+DL-2) relative to beetles feeding on less suitable plant species. For example, there was a drastic reduction in mean total pheromone (DL-1+DL-2) released from 1246 ng when males were fed on wheat to about 60 ng when fed on elm twigs. These findings and observations that beetles feeding on suitable food sources attracted more *R. dominica*, compared to those feeding on less suitable hosts, suggests that the

total quantities of pheromone (DL-1+DL-2) released, rather than modifications of ratios in pheromone blend as suggested by Bashir (2000), may be a key aspect of pheromonal signals determining the success of aggregation behavior in *R. dominica.*

Body size is known to influence reproductive success in several species of insects (Thornhill and Alcock 1983) and more specifically, can affect pheromone synthesis (Pureswaran and Borden 2003, Byers 2005). However, results from the current study indicate no relationship between *R. dominica* body size and any pheromone characteristics: quantity of DL-1 or DL-2 individually, total quantity of DL-1 and DL-2, or the percentage of DL-1 in the pheromone blends (chapter VI). We concluded that host plant, rather than beetle body size, significantly affects pheromone release by *R. dominica*.

In general, models describing the limits of flight activity under different climatic conditions will aid in the management of stored grain by predicting when migrant insect pests are a threat. Flight activity models developed in the present study (Chapter III) accurately predict when no flight activity of *R. dominica* would occur in both types of habitats, as well as when large flights (>500 near grain elevators; >220 in forested areas) would occur over short time periods (i.e. weekly interval). These models could assist farmers and grain elevator operators in predicting the onset of insect problems and aid in proper timing of management practices such as fumigant insecticide application or aerating grain bins, which are most effective after pest immigration has ceased.

A unique feature of this study is that it represents the first attempt to use diverse plant species (grain and non-grain) as food sources to investigate the nutritional ecology of *R. dominica* in relation to the influence of host plant on host location. The observation

that *R. dominica* was able to reproduce and also produce aggregation pheromones on non-cereal crops such as cowpeas, dried potato tubers and acorns underscores the potential challenges in management of this pest, as these plant species could act as alternate hosts in the absence of preferred hosts like wheat and/or maize.

References

Bashir, T. 2000. Pheromone communication and host finding behavior of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Ph.D. dissertation, University of Greenwich, U.K.

Borgemeister, C., K. Schaefer, G. Goergen, S. Awande, M. Setamou, and H. -M. Poehling. 1999. Host-finding behavior of *Dinoderus bifoveolatus* (Coleoptera: Bostrichidae), an important pest of stored cassava: The role of plant volatiles and odors of conspecifics. Ann. Entomol. Soc. Am. 92: 766-771.

Byers, J. A. 1995. Host tree chemistry affecting colonization in bark beetles, pp 154-213. *In* R. T. Cardé, and W. J. Bell (eds.), Chemical ecology of insect 2. Chapman and Hall, New York. NY.

Byers, J. A. 2005. A cost of alarm pheromone production in cotton aphids, *Aphis gossypii*. Naturwissenschaften. 92: 69-72.

Fadamiro, H. Y., I. Gudrups, and R. I. Hodges. 1998. Upwind flight of *Prostephanus truncatus* is mediated by aggregation pheromone but not food volatiles. J. Stored Prod. Res. 34: 151-158.

Linsley, E. G. 1944. Natural sources, habitat, and reservoir of insects associated with stored products. Hilgardia 16: 187-224.

Mathew, G. 1987. Insect borers of commercially important stored timber in state of Kerala, India. J. Stored Prod. Res. 23: 185-190.

Potter, C. 1935. The biology and distribution of *Rhyzopertha dominica* (Fab.). Tran. Royal Entomol. Soc. 83: 449-482.

Pureswaran, D. S., and J. H. Borden. 2005. Primary attraction and kairomonal host discrimination in three species of *Dendroctonus* (Coleoptera: Scolytidae). Agric. For. Entomol. 16: 765-782.

Pureswaran, D. S., and J. H. Borden. 2003. Is bigger better? Size and pheromone production in the mountain pine beetle *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). J. Insect Behav. 16: 765-782.

Thornhill, R., and J. Alcock. 1983. The evolution of insect mating system. Harvard University Press, Massachusetts.

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Doctor of Philosophy

Dissertation: STUDIES WITH THE AGGREGATION PHEROMONES OF *RHYZOPERTHA DOMINICA* (COLEOPTERA: BOSTRICHIDAE): HABITAT AFFINITIES, SEASONAL FLIGHT ACTIVITY, AND PHEROMONE-MEDIATED HOST SELECTION BEHAVIOR

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Title of Study: STUDIES WITH THE AGGREGATION PHEROMONES OF *RHYZOPERTHA DOMINICA* (COLEOPTERA: BOSTRICHIDAE): HABITAT AFFINITIES, SEASONAL FLIGHT ACTIVITY, AND PHEROMONE-MEDIATED HOST SELECTION BEHAVIOR.

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Major Field: Entomology

Scope and Method of Study: Two approaches were adopted in these studies. The first part of the dissertation investigated seasonal abundance and flight activity patterns of *R. dominica* in different habitats in central Oklahoma using synthetic pheromones. The second part of the study investigated host effects on reproductive success, pheromone production and host finding by *R. dominica*.

Findings and Conclusions: *R. dominica* populations were greater near grain storage facilities than in forests or in open fields, indicating the relative ability of these habitats to sustain these beetles. Captured *R. dominica* in pheromone-baited traps were female-biased, and the proportion of females to males did not differ among habitats in which the beetles were trapped. Seasonal flight activity patterns of *R. dominic*a were similar between grain elevator and forest habitats; however, in two of the three years of trapping, flight activity generally began at least 1-2 weeks earlier in forest sites as opposed to grain elevators. Weekly weather variables at grain storage elevators and forest sites, respectively explained about 80 and 86% of the variability in *R. dominica* trap captures. Behavioral responses of adult *R. dominica* showed that both sexes were able to respond to plant volatiles at short range (walking bioassay), and the attraction was strongest to plant species most suitable for beetle development. However, plant volatiles alone failed to attract dispersing *R. dominica* under field conditions. In contrast, beetles responded strongly to traps baited with male-infested plant materials, indicating that the male-produced aggregation pheromones may be the most important stimuli for host finding and subsequent aggregation in *R. dominica*. Regression analyses showed no correlation between beetle size and pheromone output in *R. dominica*. Host plant, rather than beetle body size, significantly affects pheromone release. More pheromones were released by adults feeding on plant parts such as cereal grains compared to non-cereal grains or woody tissues.

ADVISER'S APPROVAL: Dr. Thomas W. Phillips